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PHYTOPATHOLOGY

VOLUME XI NUMBER 3

MARCH, 1921

THE RESISTANCE TO CITRUS CANKER OF CITRUS NOBILIS AND A SUGGESTION AS TO THE PRODUCTION OF RESISTANT VARIETIES IN OTHER CITRUS SPECIES

FORMAN T. MCLEAN, and H. ATHERTON LEE[1]

WITH ONE FIGURE IN THE TEXT

Field observations upon the degree of citrus canker affection of the citrus species at Lamao, Philippine Islands, during the year 1917 revealed some interesting characteristics in regard to citrus canker infection of the horticultural varieties of the mandarin orange (*Citrus nobilis* var. *deliciosa*). These varieties usually showed a few cankers to each tree but the infections never became numerous and the trees were never seriously affected. The mandarin varieties have been considered by Wester (8, p. 156) to be truly resistant, and Stirling (5, p. 49) has made a similar statement for the Castalo tangerine; the junior author (3) has also presented such conclusions for the mandarin varieties. The observations and experiments here reported confirm this resistance. They also help to explain the nature of the resistance and suggest a means of utilizing it.

The infections upon the foliage of these varieties were in a great number of cases coincident with visible spine punctures or insect injuries. Since infection seemed to result only when the organism entered through some wound, the theory was formulated that the resistance of the mandarin varieties was due to some character of the epidermis. The following experiments were undertaken in an endeavor to test this hypothesis.

[1]For the laboratory facilities for this work the authors wish to thank Mr. Elmer D. Merrill, Director of the Philippine Bureau of Science. For the orchard facilities, thanks are also extended to Mr. Silverio Apostol, Chief of the Division of Plant Industry, Philippine Bureau of Agriculture, and Mr. F. G. Galang, Superintendent in charge of the Lamao Horticultural Station. Credit for the photographs is due the Philippine Bureau of Science.

An actively growing twig of Oneco mandarin was selected. The five leaves nearest the tip on this twig were coated on both upper and lower surfaces, with an infusion of *Pseudomonas citri* Hasse in tap water. This coating was made by drawing back and forth a platinum loop previously immersed in the infusion. The five leaves immediately below, on the same twig, were then coated in the same way and ten needle punctures made in each leaf through the coating of the infusion. After the inoculation moist cotton was placed around the twig bearing the inoculated leaves, and the whole twig was wrapped in strong waxed paper, to maintain a humid atmosphere around the inoculated leaves and to protect the infusion from being washed off by the intense rains. The results were: inoculations 1 to 5, without puncture, all negative; inoculations 6 to 10, each leaf with 10 punctures, all positive. As a preliminary test this corroborated the field observations very well. The experiment was therefore repeated several times on the Oneco variety, and also on the Canton mandarin, Kishiu, Szinkom, and a native seedling, using the same methods as in the preceding series unless otherwise noted. Inoculations 36 to 45 were control inoculations made on *Citrus maxima* to show the condition of the cultures of *Pseudomonas citri* which were used. They gave positive results.

INOCULATIONS ON THE HORTICULTURAL VARIETY, ONECO OF CITRUS NOBILIS VAR. DELICIOSA

Inoculations 46 to 50 and 56 to 62 made on November 20, 1917, on both upper and lower surfaces of leaves without punctures; results, 46 and 47, one infection each, evidently at a wound; remainder negative.

Inoculations 51 to 55 and 63 to 70 made on November 20, 1917, on the upper surfaces of leaves each with 20 punctures; results, positive, except 63 and 70, where the leaves dropped off. Inoculations 56 to 70 are shown in figure 1.

Inoculations 96 to 101 made on December 5, 1917, on upper surfaces of leaves each with 20 punctures; results, 4 leaves 100 per cent positive, 2 leaves 95 per cent positive.

Inoculations 102 to 106 made on December 5, 1917, on both upper and lower surfaces of leaves without punctures; results, negative.

INOCULATIONS ON THE HORTICULTURAL VARIETY, SZINKOM OF CITRUS NOBILIS VAR. DELICIOSA

Inoculations 21 to 25 made on November 19, 1917, on upper surface of leaves without puncture; results, all negative.

Inoculations 26 to 30, November 19, 1917, on lower surface without punctures; results, all but 29 negative; 29 positive, apparently because of insect injuries through which infection occurred.

Inoculations 71 to 73 and 79 to 83 on November 28, 1917, on both upper and lower leaf surfaces without punctures and not protected with paraffin paper; results, negative except for one canker.

Inoculations 74 to 78 on November 28, 1917, on upper surface of leaves with 20 punctures on each leaf and not protected with paraffin paper; results, from 5 per cent to 75 per cent positive.

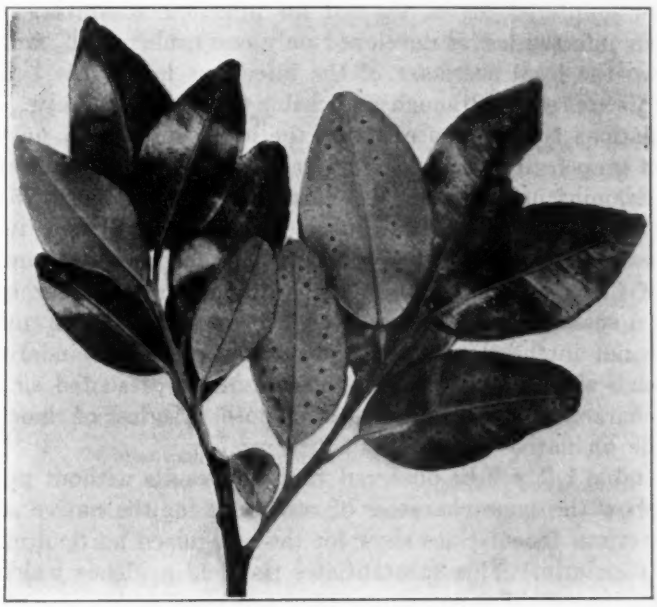

Fig. 1. Inoculated Leaves 56 to 70, "Oneco" Orange

Twig on left, leaves coated with infusion of *Pseudomonas citri* without wounding. Twig on right, leaves treated in same way but 20 punctures made in each leaf.

Inoculations on the horticultural variety, Kishiu of Citrus nobilis var. deliciosa

Inoculations 84 to 88 made on November 28, 1917, on the upper surfaces of leaves each with 20 punctures; results, 3 leaves 100 per cent positive and 2 leaves 95 per cent positive.

Inoculations 89 to 92 made on November 28, 1917, on both upper and lower surfaces of leaves without punctures; results, 3 leaves negative, 1 leaf dropped off.

Inoculations 107 to 111 made on December 6, 1917, on upper surface of Mandarin leaves each with 20 punctures, gave 100 per cent positive results.

Inoculations 112 to 116 on both upper and lower surfaces of Mandarin leaves without punctures were entirely negative.

To summarize: forty-eight leaves were inoculated without punctures on six different days during a period of two and one-half months, all but seven of which were negative—*i. e.*, Szinkom Nos. 29 and 72, Oneco Nos. 46, 47, 49, 50, and 105. Of the seven infected leaves three showed infections only at very evident wounds. Thus forty-one of the forty-five uninjured leaves showed no infection whatever. Two of these four infected leaves developed only one canker each, and on the other two the local character of the infections led to the belief that wounds were present although not visible to the unaided eye.

Inoculations 1 to 5 were made on the lower surfaces of unwounded leaves of grapefruit, *Citrus maxima* var. McCarthy, on November 19, 1917. Examination six days later showed 15 cankers in no. 2, 25 cankers in no. 3, 38 cankers in no. 4, 5 cankers in no. 5, and none in no. 1. This shows clearly the small proportion of infections on unwounded leaves of *Citris nobilis*, as contrasted with the large number of infections on a susceptible species such as *C. maxima*. These numerical data are not intended to prove the resistance of the mandarin varieties which is already generally recognized, but are presented as indicating the character of their resistance. Another series of inoculations was made on native seedlings of *Citrus nobilis*.

From table 1 it will be observed that the results without punctures do not show the same character of resistance for the native seedlings as the previous inoculations show for the recognized horticultural varieties of mandarin. This substantiates the field evidence which shows that the native Philippine mandarin orange trees, although varying in their susceptibility to canker, are in almost all cases more readily attacked than are the commonly propagated horticultural mandarin varieties.

The results of these experiments seem to warrant the following conclusions: (1) That the horticultural varieties of the mandarin orange (*Citrus nobilis* var. *deliciosa*) are truly resistant to citrus canker except when wounds permit the entrance of the causal organism. (2) That when punctures through the epidermis occur, these varieties give as large a proportion of positive results as do inoculations on *Citrus maxima* or *Citrus aurantifolia* (the most susceptible species). (3) Apparently, then, the epidermis is the structure which renders *Citrus nobilis* resistant to canker.

TABLE 1

Inoculations on Philippine seedlings of Citrus nobilis, date of inoculations, January 4, 1918, date of observation of results, January 31, 1918.[2]

INOCULATION NUMBER	METHOD	RESULT
151	Upper and lower surfaces without punctures.	Shows 2 cankers
152	Do	Shows 2 cankers 1 at evident wound
153	Do	Many small infections at tip.
154	Do	Many small infections Many at evident wounds.
155	Do	No cankers.
156	Do	Leaf used by ants in making nest, lost for observation.
157	Upper surfaces only with 20 punctures.	100% positive
158	Do	90% "
159	Do	100% "
160	Do	90% "
161	Do	100% "
162	Upper and lower surfaces without punctures.	Negative
163	Do	Do
164	Do	Do
165	Do	Do
166	Do	Do
167	Do	Do
168	Upper surfaces only with 20 punctures.	80% positive
169	Do	60% "
170	Do	60% "
171	Do	80% "
172	Do	60% "

A preliminary examination of the epidermis of resistant mandarin orange and non-resistant grapefruit leaves showed some differences, but none of these appear to explain the resistance. The structure of the epidermal tissues of *Citrus maxima* is much looser and the stomata are more numerous than those of *Citrus nobilis;* 38 stomata per square millimeter were found on *Citrus nobilis* and 50 per square millimeter on *Citrus maxima.*

The size of the stomata in these two species, however, was not found to be very different; the outside dimensions of those of a native form of *Citrus nobilis* are 17.5×17.0 microns and those of the native pummelo (*Citrus maxima*) are 20.0×19.0 microns. No apparent difference

[2]All of the inoculated twigs have been preserved as herbarium specimens and may be examined by any one interested in the results.

has been observed in the structure or size of the stoma such as would account for the exclusion of microorganisms of the size of *Pseudomonas citri*. Hasse (2, p. 99) gives the size of these organisms as 1.5 to 2.0× 0.5 to 0.75 microns.

Since the resistance appears to be confined to the epidermis, the authors propose that this fact be utilized in attempting to develop canker-resistant varieties of other Citrus species. It will be recalled that, in 1908 and 1909, Winkler (9), (10), (11), (12), working with species of Solanum succeeded in obtaining, by grafting, branches in which the tissues of two species were intermingled. These were of two types: one had sectors of the axis of different species and the other type had the separate layers of tissues of different species. These latter are termed periclinal chimeras or "graft hybrids" and those produced by Winkler all showed that the fruit character is controlled by the internal tissues, being little modified by the epidermis. Similar chimeras were also described by Baur (10), (1), with Pelargoniums. Sectoral chimeras (of the first type) have been produced repeatedly in Citrus, and have been perpetuated in Italy. These have been investigated quite thoroughly by Strasburger (6).

The second type has not been noted in Citrus, but the existence of several forms of sectoral chimeras leads to the belief that periclinal chimeras can be developed also, with epidermis of a resistant type of *Citrus nobilis* and the internal tissues of desirable varieties of *Citrus maxima*. Such dual structures exist in other woody chimeras as McFarlane (4, p. 268) has shown.

A periclinal chimera possessing the resistant epidermis of *Citrus nobilis* would appear to be protected from canker to the same degree as are the mandarin orange varieties of *Citrus nobilis*. The results of Winkler with Solanum indicate that the fruit characters would be controlled by the internal tissues and therefore would be very nearly like those of the *Citrus maxima* parent. The peculiar structure of citrus fruits does, however, modify this general statement somewhat. The pulp vesicles of the sections as shown by Tschirch and Oesterle (7, p. 303, pl. 70) are epidermal outgrowths of the walls of the section, and the membrane surrounding each vesicle is true epidermis, the juicy portion of each vesicle being of internal tissue. Thus the fruit of a chimera such as is proposed would probably have its texture but not its flavor modified by the character of the epidermal tissue. Since the known commercial grapefruit varieties are extremely susceptible, a periclinal chimera possessing the internal tissue of the grapefruit, with the epidermis of the mandarin, would appear to offer one solution to the problem of securing canker-resistant grapefruits.

SUMMARY

(1) Field observations on the occurrence of citrus canker on the horticultural varieties of *Citrus nobilis* var. *deliciosa* suggest that infections are in a large proportion of cases coincident with visible wounds.

(2) This conclusion was substantiated experimentally, the results showing that these varieties were fully as susceptible as the grapefruit varieties, when an entrance to the tissue was made for the parasite.

(3) The suggestion is presented that the periclinal chimera in which the epidermis is of *Citrus nobilis*, might confer resistance on other very susceptible but desirable Citrus species, while at the same time the character of the fruit would be but little changed.

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OBSERVATIONS ON WHEAT SCAB IN PENNSYLVANIA AND ITS PATHOLOGICAL HISTOLOGY

J. F. Adams[1]

With Plates II and III and One Figure in the Text

The prevalence of wheat scab or Fusarium blight of wheat was early reported in this country by Weed (11) in 1890, Chester (3) in 1890, Arthur (1) in 1891, Hickman (5) in 1892, Selby and Manns (10) in 1909 and others. Chester (3) in Delaware states that it is a "serious disease and frequently occasions not more than one-half of a normal yield on account of the shriveling grain." Weed (11) in discussing wheat scab says: "In 1890 I saw a field of one hundred acres in Madison County, Ohio, considered the finest wheat field in the country, which was expected, shortly before harvest, to yield thirty-five to forty bushels per acre, so severely attacked by the disease that the yield was reduced to eight bushels per acre. Two other fields, one of twenty-five and the other of fifty, were shrunken in yield at least one-third." Arthur (2) in 1898 reported infection for Indiana ranging from 25 to 75 per cent. Johnson, Dickson and Johann (7) have recently reported infection ranging from a trace to fifty per cent for the Mississippi Valley and eastward.

As with the above reports on the prevalence of wheat scab the observations in Pennsylvania have been confined to the infection on the head. While no doubt seedling infection is a stage of the disease directly affecting the yield it has been impossible to ascertain the loss in this respect with any certainty. The highest estimate of scab infection in wheat fields under our observations has been 8 per cent. In 1917 the average infection for the state was estimated at 2 per cent. Since the average infection under our conditions involves such a small portion of the head the infection in the aggregate cannot be considered as a serious loss. It is conservative to state that the disease is present in all the wheat-growing centers of the state. Figure 1 indicates the distribution of wheat scab as shown by the plant disease survey reports for the state according to counties during the three-year period 1917–1920.

[1] Contributions from the Department of Botany, The Pennsylvania State College, No. 29.

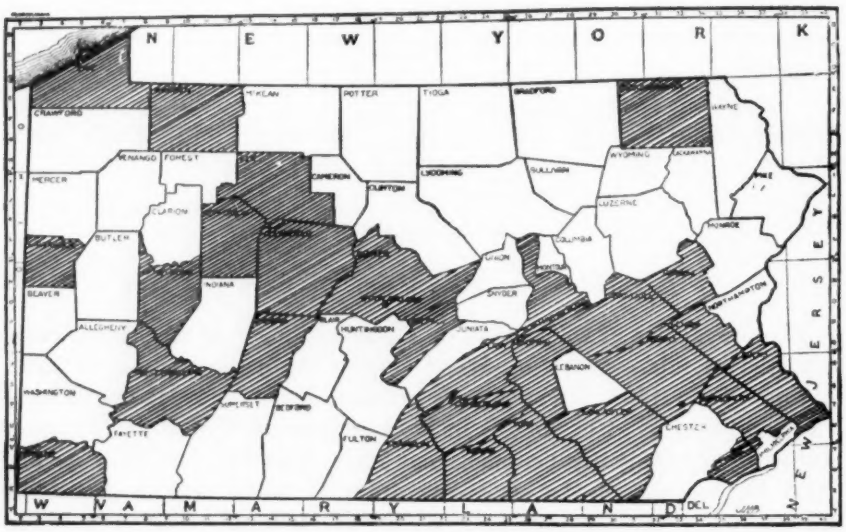


FIG. 1. Occurrence of wheat scab caused by *Gibberella saubinetii* in the counties of Pennsylvania during the years 1917 to 1920 is indicated by the crosshatching.

Field observations as reported by Hoffer, Johnson and Atanasoff (6) and others, as confirmed by our observations in this state, show a greater prevalence of wheat scab in fields where in crop rotation wheat has followed corn. One commonly finds on the old corn stubble in spring the fruiting bodies of *G. saubinetii* which no doubt are in part the source of inoculum. In some instances the fruiting bodies of *G. acervalis* are found associated with *G. saubinetii*. It is a common practice to only disk and harrow corn fields for sowing wheat in which case numerous stalks are exposed. In this way wheat seedlings as well as the head are subject to infection by the fungus on the exposed corn stalks. The loss through seedling infection is difficult to estimate, but our attention has not been called to any prevalent dying out in wheat fields where *G. saubinetii* could be attributed as the cause. Inoculations with this parasite on seedlings in moist chamber as well as in sterilized soil have established its virulent ability to kill seedling wheat.

Numerous predisposing factors have been reported favoring wheat scab infection. Weed (11) observed it as worse on weak growing varieties and those fields which are sowed latest. Hickman (5) states that defective drainage evidently increased the scab on the station farms and, in short, everything which tends to weaken the plant or retard its ripening renders it more subject to an attack. Arthur (2) observed that the attacks of the scab fungus were worse upon the late maturing than the early ones and certain French varieties were par-

ticularly susceptible. Selby and Manns (10) found that wheat scab was more severe on unmanured soil and on continuous planted areas. It was also determined that the scab fungus in the unfertilized plots destroyed an average of 5.9 per cent plants, while the fertilized plots showed 3.7 per cent. In rotation fields this loss was from 1 to 1.5 per cent. Mortensen (8) in his extensive review and studies of cereal diseases caused by Fusarium spp. states that "the dependence of the Fusarium attacks upon the weather conditions, damp air, and altogether abundant moisture favors without doubt the growth of the species of Fusarium and presumably all of these." He observed that infection was especially prevalent in wet years usually occurring between the time of flowering and maturing of the cereals.

Wheat scab infection as found under our conditions is usually restricted to one or two spikelets of the head. However, one occasionally finds the upper half or the complete head infected. I have first observed it in the "milk" or "setting" stage when the glumes of the infected spikelets appear as ripening prematurely. At this time it can be recognized by the chlorotic appearance of the glumes on infected spikelets. When the head is ripened the disease is not so easily recognized unless the fungus is fruiting abundantly. Weed (11) states that it is usually first noticed just as the heads are beginning to turn, when an examination of infested fields shows that with a portion of the heads the upper or lower half has prematurely whitened, leaving the rest green, the whitened part having on many of the glumes a more or less distinct or orange covering of the mycelium and the spores of the fungus. Detmers (4) reports that the whole ear may be diseased, but more commonly the upper, lower or central portion only is affected, and quite often the disease is confined to a single spikelet, the adjoining ones being apparently healthy.

The glumes of infected spikelets are usually closed and appear to be held together by the fungus growth within. In advanced stages of infection a considerable portion of the fungus growth is evident where the glumes overlap and in extreme cases the outer surface is covered with the interlaced mycelium. At the base of the glumes the superficial pink growth of the fungus is evident. Where a dense growth of the fungus is found it is most easily recognized by its dominant pink or salmon color. Upon separating the glumes of the infected spikelets one usually finds abundant superficial growth of the fungus. The kernel of wheat is conspicuously shriveled and often covered with a dense growth of mycelium usually white in appearance. Associated with the superficial fungus growth we find the typical spores of *G. saubinetii*.

Material of wheat scab from the West identified as caused by *G. saubinetii* is similar to our infection observed in Pennsylvania. With the severe outbreak of scab last year in the middle west a systemic infection of the head was common. Mr. H. W. Thurston, Jr., informs me that infection involving a half of the head is more common than under our conditions in Pennsylvania. Material with the perfect stage of *G. saubinetii* has been examined in which half the spikelets were infected and conspicuous with the black perithecia distributed over the glumes. We have not collected in Pennsylvania any material with the perfect stage of *G. saubinetii* fruiting on wheat.

All material which I have observed since 1914 in the state has shown that mature kernels were apparently infected, although it was commonly observed with some of the western scab that undeveloped ovules had become infected. Weed (11) states that the fungus apparently gains access to the tender undeveloped kernel. This difference is unquestionably dependent upon the time of infection.

The cause of wheat scab has been attributed to different species of Fusarium and Giberella by various investigators. The early references to wheat scab usually associated *Fusarium roseum* and *F. culmorum* as the cause. Wollenweber (12) has stated that *Giberella saubinetii*, *F. culmorum*, *F. subulatum* and *F. metochrorum* were formerly included in the collective species *F. roseum*. Owing to the early confusion in defining species of Fusarium and the similarity in signs of the fungus as described by various investigators, it is impossible to determine with certainty that *G. saubinetii* was the causal organism. Selby and Manns (10) were among the first in this country to prove conclusively the relation of *G. saubinetii* to wheat scab. These authors reported their cultures of *F. roseum* were identical with *G. saubinetii*.

Extensive culture work on this disease by Johnson, Dickson and Johann (7) has shown that three species are to be associated with this disease. "An extensive survey over fifteen states, correlated with an intensive survey over nine states in the winter-wheat area, brought out some pertinent facts with reference to the epidemic. A careful study of over one thousand specimens collected in fifteen states showed *Gibberella saubinetii* (Mont.) Sacc. to be the chief causal organism. Less than 1 per cent of the specimens yielded *Fusarium culmorum* (W. Sm.) Sacc., *F. Avenaceum* (Fr.) Sacc. and other Fusarium species."

Cultures of wheat scab from various sections of Pennsylvania have consistently identified *Gibberella saubinetii* as the cause. Typical conidia and absence of chlamydospores along with cultural reactions on cellulose and various sugar media have sharply differentiated this species from *G. acervalis* and *F. culmorum*. One strain of *G. saubinetii* which was

isolated from corn by Mr. F. G. O'Donnell has shown the tendency to produce perithecia, but no culture from wheat has developed perithecia.

The pathological histology of infected kernels has been given little study by previous workers. Chester (3) has stated "that a microscopic examination of the seed when badly shrunken shows it to be completely replaced by mycelium. The threads of this mycelium penetrate every portion of the grain, even to the germ, thus destroying its vitality." Arthur (1) reports that scab in wheat heads "is due to a minute fungus that attacks the wheat heads at the time of flowering. The spores of the fungus blow through the air, lodge on the delicate parts inside the flower and soon penetrate the kernel and envelope it with a mesh of moldy filaments which sap the life of the kernel and forming new spores spread the disease to other flowers throughout the field." Detmers (4) reported "the affected grain is very much shrunken and is covered externally by a thick white felted mass of mycelium of the fungus. On cutting open the kernel it is found to be hollow and filled with white mycelium." Weed (11) observed that "the kernels attacked by the fungus become mere shells covered inside and out with mycelium."

Material for study was obtained from infected ripened heads in the field. The shriveled kernels were soaked in water for twenty-four hours and then fixed for the same length of time in Petrunkewitsch killer. It was dehydrated by the usual method and kept in paraffin bath for forty-eight hours. Sections were cut 8 to 10μ in thickness and stained with Flemming's triple stain. Serial cross sections were made of infected kernels in order to determine the character of infection from the germinal end to the crown.

In infected as well as normal kernels the entire tissue of the pericarp or ovary wall is partly lacking since this in part becomes membranaceous and sloughed off with maturity of the kernel. The mycelium is found to have an extensive ramification throughout the kernel being intracellular as well as intercellular and all parts are penetrated. The integuments and nucellus were almost intact. The peripheral mycelium was often observed penetrating the cells making up the integuments. Typical appresoria-like bodies were formed as the means of intracellular penetration of the integumental cells, but this always appeared to be subsequent to the establishment of internal infection. The intracellular mycelium fills the cells like a number of haustoria and ramifies from cell to cell.

The nucellus was seldom disorganized and appeared to be the limiting factor in confining the fungus development within the seed. The

fungus evidently develops to the greatest extent within the limits of the nucellus.

All evidence would indicate that the fungus apparently gains entrance through the germinal end and after working inwards eventually develops towards the periphery. The development of the fungus in the glumes and cells external to the nucellus would seem to be subsequent to primary infection of the kernel.

In cross sections of infected kernels the protein or aleurone layer was found engulfed on either side by parallel strands of mycelium. This parallel mass of hyphae is most conspicuous between the nucellus and aleurone layer. The large cells making up the aleurone layer are early penetrated by the mycelium. These cells being rich in stored food seem favorable for abundant development of the intracellular mycelium. After the cellular contents are used up by the fungus the cells collapse and with advanced infection no evidence of the aleurone layer remains. The space usually occupied by this layer of cells is now replaced with extensive development of the mycelium. In some instances typical massing of the hyphae occur as shown in plate III, figures 3 and 4. These masses of interlaced mycelium have the appearance of incipient sporodochia although no mature ones were found developing spores. Their development was towards the periphery and approximate to the inner part of the nucellus.

No indication of the endosperm tissue was present in the infected kernels. The large storage cells for starch are not in evidence and in the space usually occupied by the storage cells only a loose irregular mass of starch grains remain as shown in plate III, figure 3. In some instances starch was found to be entirely absent. The complete disorganization and disappearance of the endosperm tissue establishes the evidence of a lysigenous effect subsequent to infection by this parasite. Long irregular branching hyphae are found in the endosperm cavity but not conspicuous as adjacent to the nucellus.

The most outstanding effect of this fungus is found at the germinal end in the embryo. The affinity this fungus has for the embryonic tissue is very striking and is well supported by the results secured with inoculations. There was no evidence of embryonic tissue in the infected kernels I have examined that were collected in the field, as it had been entirely destroyed as the result of infection. In the area usually occupied by the embryo there is found a compact mass of interlaced mycelium as shown in plate II, figure 2. How far the embryo was developed at the time of field infection could not be ascertained. However, with the size and development of other parts of the kernel as compared with normal kernels it would appear to have been ma-

ture. It is probable that infection becomes established first with the developing embryo since this is so favorable for the fungus development, and then spreads to the storage or endosperm tissue. As observed with the endosperm tissue the lysigenous effect on the embryonic tissue as the result of infection is very evident. It was also noted that the aleurone, nucellus and outer integuments in normal kernels were not as well developed for protection of the embryo as at other parts of the kernel.

The effect of this parasite on the glumes and rachis was very restricted; possibly associated with the greater cellular resistance. Cross sections of the glumes showed penetrating hyphae as well as disorganized cells. In some instances certain cells were filled with intra-cellular mycelium but not so extensively as with the kernels. Sections of the rachis showed very slight infection in the material studied. There were no indications that it progressed in this tissue from spikelet to spikelet.

Inoculation experiments were conducted to determine further the virulence of this fungus under artificial conditions. Durtz Longberry Red and Poole varieties were used in the experiments. The wheat was disinfected for three minutes in 1–1000 solution of $HgCl_2$ and then placed on moistened filter paper in sterilized chambers. Various lots of ten seeds after twenty-four hours and up to three days germination were removed to other sterile moist chambers, inoculated and kept under room temperature conditions of 22° C. In all inoculations mycelium was used as the inoculum from cultures of *Gibberella saubinetii* isolated from wheat scab and corn root rot. The mycelium was placed on the surface of the germinal end of the embryo and all results were positive.

There was no apparent difference as to susceptibility of seedlings up to three days' growth. Within a period of six days infection was so well established as to kill the embryonic tissue. The mycelium appeared to first become established in the embryonic tissue and then the entire kernel was involved with a profuse growth of mycelium. In cases where the plumule was two or three inches long it would collapse at the base and droop as a result of infection. The roots were attacked and covered with a growth of mycelium. Material at different stages of development was killed by the method already described. It was found that fungus infection developed most rapidly at room temperature. Inoculated kernels under temperature conditions of 8–10° C. retarded infection at least three days but eventually became established. This difference is associated with the low temperature favorable for germination and a higher temperature unfavorable

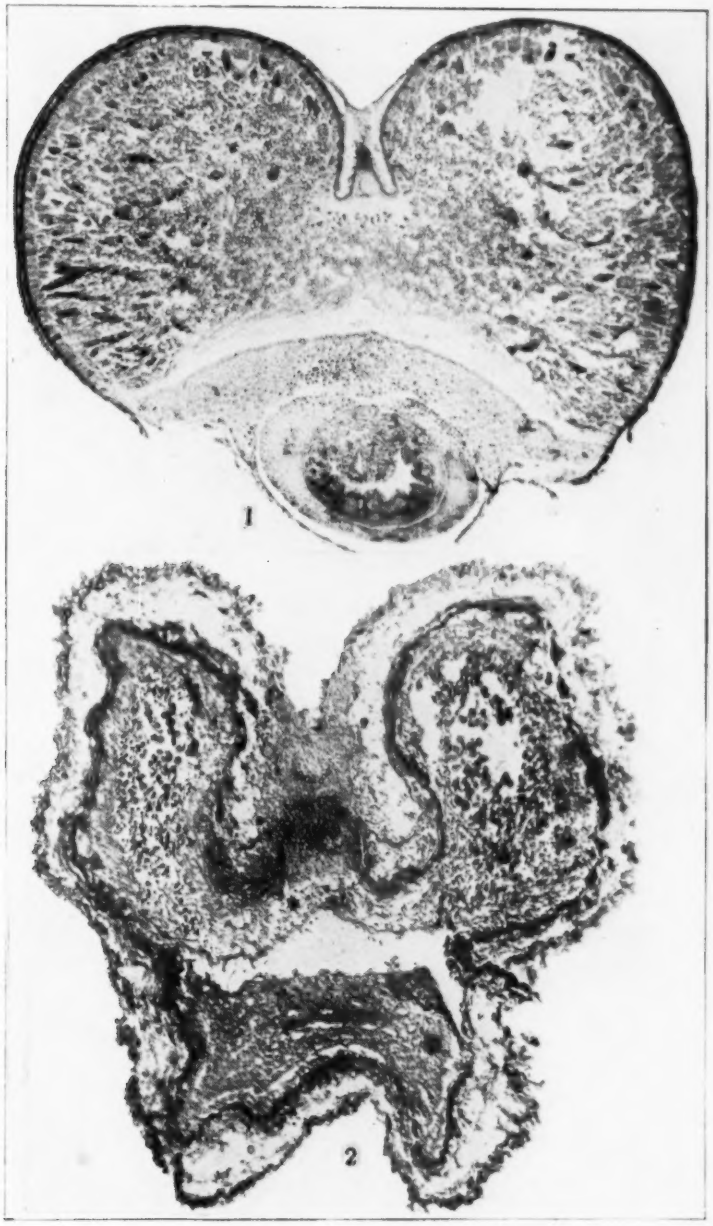
for growth of the fungus. Muth (9) observed less infection with *F. roseum* at 20° C. than with seeds at 30° C.

In the inoculated kernels the fungus becomes well established between the endosperm and scutellum. Plate II, figure 5, shows a portion of the palisade epithelium and scutellum storage tissue after the sixth day of inoculation. An entire cross section shows a weft of mycelium completely surrounding the scutellum. The epithelial cells are conspicuously plasmolysed and at a later stage the cells are penetrated and the tissue disorganized. Plate II, figure 5, shows the progress of the fungus at the end of six days in its characteristic massing of mycelium between the nucellus and aleurone layer as observed with the infected kernels from the field.

The root system is as severely attacked as the embryo. The cortical tissue as well as the stele appears to offer no resistance to the extensive ramification of the fungus. In cross sections of secondary roots the stele was found completely disorganized and replaced with strands of parallel hyphae.

Disinfected wheat after 48 hours in moist chamber was inoculated as previously mentioned and planted one-half inch in depth in sterilized soil. The same varieties of wheat were used as in the moist chamber inoculations. They were watered each day with distilled water and kept under room temperature conditions and exposed to window light. The control pots showed normal growth for the period of eighteen days at which time the plumules or seed leaves in the inoculated pots had died down. Good growth was made for the first four days in the inoculated pots but then appeared to gradually weaken and were finally killed. Examination of the seedlings showed the same disorganization of tissue as observed with the inoculations in moist chambers. The only difference noted was the interval of time with respect to the establishment of infection which would suggest that under soil conditions the infection period is prolonged several days due to lower temperature.

The evidence from inoculation experiments under these conditions fully supports the results observed with infected material under field conditions. As a further check inoculations were carried out on wheat heads in the "flowering" and "milk" stage. The inoculum used was the same as with the seedlings and placed on one or two spikelets of a head and the entire head protected in a glassine bag. At the end of twelve days the heads were cut and examined. Positive infection was secured in all but three inoculations of thirty heads infected. The infection in every instance was confined to the point of inoculation. There was no indication of its spreading throughout the head. Infections in the flowering stage showed the young ovules never matured



CROSS SECTIONS OF NORMAL AND DISEASED WHEAT GRAINS

but were reduced to a small shriveled mass consisting for the greater part of mycelium. There was no conspicuous growth of superficial mycelium inside or upon the glumes. The infected spikelets appeared similar to the condition called "blasting of heads." Infection in the milk stage produced shrivelled kernels as found under field conditions. The shrivelled kernels and the glumes were covered with a conspicuous development of mycelium. The embryo was found disorganized as observed under conditions of natural infection in the field.

CONCLUSIONS

Wheat scab in Pennsylvania has been observed to be most prevalent as an infection of the head. While seedling infection is no doubt a serious phase of the disease it is probable that soil and temperature conditions with the planting of wheat in the fall are not conducive to seedling infection.

Wheat scab is more serious in fields following the planting of corn and the evidence is quite convincing that in part the source of inoculum comes from the development of *G. saubinetii* on corn stalks.

Observations of infected and inoculated kernels indicate that the fungus if once established will destroy the embryo.

Inoculation experiments support the evidence that under favorable conditions *G. saubinetii* may become a virulent parasite of wheat seedlings.

A study of infected kernels from various parts of the state collected at the time of harvesting indicate that the prevalent type of infection occurs after the flowering stage.

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PLATE II. CROSS SECTIONS OF NORMAL AND DISEASED WHEAT GRAINS

Fig. 1. Photomicrograph of a cross section of a normal wheat kernel through the germinal end. This illustrates the normal appearance and position of the pericarp, aleurone, endosperm and embryonic tissue.

Fig. 2. Photomicrograph of a cross section through the germinal end of a wheat kernel infected with *Gibberella saubinetii*. The complete disorganization of the above mentioned tissues is in sharp contrast. The aleurone layer and endosperm tissue is completely disorganized. The embryonic tissue is practically replaced by an interlaced mass of mycelium. No evidence of the scutellum is to be found.

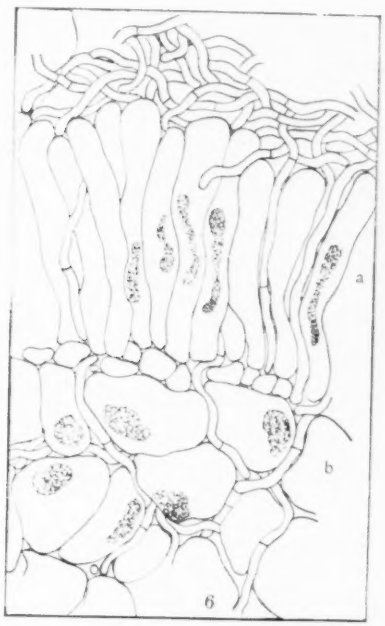
PLATE III. HISTOLOGY OF WHEAT SCAB.

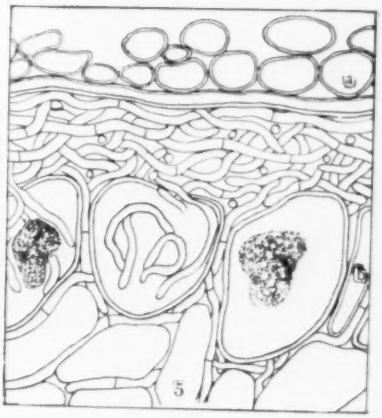
Fig. 3. Photomicrograph of a cross section above the germinal end of a wheat kernel infected with *Gibberella saubinetii*. The endosperm tissue and aleurone layer appear the same as in figure 2.

Fig. 4. Photomicrograph showing an enlarged view of a portion of the periphery of figure 3. The large dark globular masses are the sporodochia which originate between the nucellus and aleurone layer.

Fig. 5. Portion of the periphery of a kernel six days after inoculation with *G. saubinetii*. The contents of the aleurone layer have collapsed and the two cells on the left show penetration of the mycelium. The extensive development of the mycelium is shown as it developed like a wedge between the nucellus (a) and the aleurone layer (b). It is in this area that the sporodochia originate.

Fig. 6. Showing portion of the scutellum after six days inoculation with *G. saubinetii*. The mycelium develops extensively between the palisade of epithelium and the endosperm and then becomes established in the storage tissue of the scutellum. The mycelium is at first intercellular and the host cells are plasmolysed but later the mycelium becomes intracellular causing complete collapse of tissue. a-palisade epithelium, b-parenchyma storage cells of scutellum.





HISTOLOGY OF WHEAT SCAB

EXPERIMENTAL DATA ON LOSSES DUE TO CROWN-CANKER OF ROSE

L. M. Massey

Some observations and experimental data concerning the pathogenicity of *Cylindrocladium scoparium* Morgan, the cause of crown-canker of rose, have been recorded by the writer in a former publication.[1] Statements based principally on observations and on reports of growers were made to the effect that diseased plants do not die quickly but linger on and yield increasingly poor and few blossoms; that they do not respond to heavy applications of fertilizers as do healthy plants; that the number of plants actually killed within the duration of time they are kept by growers is very small, but that the normal activities of the plant are so materially interfered with that they can be grown only at a financial loss; and finally, that *C. scoparium* is a fungus low in parasitism and that conditions of moisture are important factors in its development.

Since mortality can not be used as an index of the financial loss occasioned by this disease, it seemed desirable to determine by experimentation the nature and the extent of the losses. Some data indicative of the nature of the injury have been obtained in an experiment extending through three years' time. Incidentally, other facts relative to the pathogene and to experimentation under greenhouse conditions have been obtained, and are here set forth.

The house in which the plants were grown was of modern construction equipped with side and over-head ventilators. No roses had ever been grown in it. There were three solid beds, constructed of concrete, each 21 feet in length, the two outside ones being 4 feet in width (inside measurements), and the middle one 5 feet. The walls of the beds were 4 inches thick. The sub-soil in these beds was a mixture of sand and gravel. The soil obtained for growing the plants was composed of 4 parts of heavy sod composted with 1 part of manure. Before placing the soil in the beds well-rotted cow-manure was added in the proportion of one to three, mixed thoroughly and the mixture steam-sterilized. The steaming was done in a bed 4 feet in width, by placing the soil in a 12-inch layer over 4 perforated pipes running

[1]Massey, L. M. The crown canker disease of rose. Phytopathology **7**: 408–417. *3 fig.* 1917.

lengthwise of the bed. During the treatment the soil was covered with canvas. About five pounds pressure was maintained in the pipes, and the treatment lasted three hours. The house was steam-heated, the pipes carrying the steam being hung on the side walls about 16 inches removed from each of the two outside beds.

A total of 221 plants was used in the experiment, 68 of which were planted in each of the two outside beds, and 85 in the middle bed. They were of the variety Ophelia, grafted on Manetti stock.[2] As the plants increased in size following their receipt on March 1, 1917, they were shifted to 3-inch and later to 4-inch pots, using stem sterilized soil, and finally on May 21, 1917, to the beds containing the steamed soil. The beds were then divided into four sections, each, by means of new hemlock boards measuring 1 inch in thickness and 12 inches in width. The boards were sunk into the soil to a depth of about 10½ inches. The sections, with the number of plants in each, are indicated in table 1. Each of the sections A to C, and J to L, inclusive, contained 20 square feet; sections D and I, 24 square feet each; sections E, G and H, 25 square feet each; and section F, 30 square feet. The plants were 10 inches apart, crosswise of the bed, and 14 inches apart, lengthwise.

Cultural studies had given no indication of sensitiveness of the fungus to either acid or alkaline media. It was thought, however, that possibly the addition of certain chemicals to the soil might exert some influence upon infection by the pathogene, either through toxic action, by changing the acidity of the soil, or indirectly through their effect on the host. Consequently on July 17, 1917, the following chemicals were applied in the stated amounts per square foot to the indicated sections (see table 1): section A, calcium sulphate,[3] 0.1 pound; section B, potassium sulphate, 0.1 pound; section C, ammonium sulphate, 0.025 pound; section D, acid phosphate, 0.5 pound; section L, stone lime, 0.2 pound. Sections E to K received no chemicals. The dry chemicals were sprinkled over the soil, and worked in with a surfacer. On the following day the soil was well watered.

On July 20, 1917, the soil in sections A to D and J to L was inoculated as follows: Six-weeks-old cultures of *C. scoparium* growing on potato-agar in Erlenmeyer flasks of 300 cc. capacity were thoroughly mixed with a small quantity of equal parts of corn-meal and sand. A handful of this mixture was placed in the soil near, but not in contact with, each plant. An attempt was made to add approximately equal quantities of inoculum in each case. The inoculum was then

[2] The writer wishes to express his appreciation of the kindness of A. N. Pierson, Inc. of Cromwell, Connecticut, in furnishing *gratis* the plants used in this experiment.

[3] The chemicals, calcium sulphate, potassium sulphate, and ammonium sulphate, were chemically pure.

TABLE 1

Arrangement of beds and sections, and the relative positions of rose plants in each. The figures indicate the relative positions of the plants and the number of blossoms produced by each during the third season. Starred letters indicate sections containing infested soil, starred figures indicate the plants from which successful isolations of the fungus were made.

45	42	40	27	37		37	37	26*	18*		24*	27*	21*	27*		31*	19	19	23*
34	29		27	23		24*	9*	16*	9*		19*	21*	22*	25*		14*	26*	11*	27*
40	33	29	36	20		19*	34	14*	10*		26*	27	17	14		15*	19*	21*	35
59	37	43	42	30		44*	27*	13	26		28*	23	29*	22*		25	24	31	21
		I					J*					K*						L*	
19	27	41	21		23	24	36	53	43		34	33	34	39		43	34	49	39
37	23	26	19		20	30	41	33	22		40		38	31		19	16	22	31
28	24	31	25*		26	39	19*	43	30		20	48	26	37		46	25	30	33
26	26	18	24*		28	31	7*	28	30		15	28		39		31	23	31	15
29	36	24	28		34	46	41	43	35		32	49	36	42		34	37	49	29
		E					F					G						H	
32*	17*	20*	23*		22*	24	32	23		29*	22	18*	25*		17	22	23*	20*	18*
18	9	9*	10*		13*	18*	27*	16*		15	19*	27*	24		22*	16*	23*	21*	21
16*	35*	17	16*		18	22*	21	31*		21*	28	24*	24		20	21*	23*	23*	18*
17*	25*	16*	23*		29	33	21*	29		28	27	23*	29*		22	26*	21*	28*	24*
		A*					B*					C*						D*	

mixed with the upper inch or so of soil, with the aid of a surfacer, and the soil well watered. The above procedure was duplicated on January 11, 1918. Sections E to I received no inoculum.

No attempt was made to control the time of cropping by "pinching" the buds. Otherwise the methods used in handling the plants approximated those of commercial growers. The temperature of the house was held at 65° F. during the day, and 60° F. at night. The plants were rested during the summer by withholding water for about six weeks, beginning the middle of June. During the last week of July, just prior to the resumption of watering, the plants were cut-back, and the upper 3 inches of soil replaced by a mixture of soil and well rotted cow-manure in equal amounts, to which was added a small quantity of bone meal. In January and again in March of each year a top-dressing of well rotted cow-manure about 2 inches in thickness was evenly distributed over all three beds. The moisture content of the soil was not controlled. An effort was made, however, to keep the soil in all sections as uniformly moist as possible by the ordinary means at the disposal of a commercial grower, and as moist as was consistant with good growth. Although care was exercised to see that no soil was carried from one section to another, it is doubtful if the precautions taken were sufficient to entirely prevent this possibility, the sections being separated merely by thin boards. It is indeed surprising that, as indicated by culture studies (see page 129), but four of the plants growing in presumably uninfested soil became diseased.

Little black-spot (*Diplocarpon rosæ* Wolf) appeared in the house during the three seasons. This disease, together with mildew (*Sphærotheca pannosa* (Wallr.) Lév. var. *rosæ* Wor.) which appeared at various times during each winter, was readily controlled by dusting the plants with a mixture of 90 parts finely ground sulphur and 10 parts of arsenate of lead. Upon the appearance of either black-spot or mildew, all of the plants were immediately dusted. Three or four applications of dust made at weekly periods, together with the judicious use of water and the regulation of the temperature, were sufficient to control the diseases in all cases.

During the first season (winter of 1917–1918) no record of blossoms produced by the plants was taken. On December 13, 1917, the soil was pulled away from the crown of each plant in an attempt to determine whether or not the disease was present. In many instances there were unmistakable symptoms of infection; in other cases it was impossible to determine whether the darkening and checking of the tissues at the crown were due to disease or to the normal habit of the plant. Consequently no record of any value could be made.

During the season of 1918–1919 a record was taken of the number

of blossoms produced by the plants in each section, but not from individual plants, as was done during the season of 1919–1920. The records are shown in tables 1 and 2. Throughout the three years in which the plants were grown not a single plant died from crown-canker or any other cause. Three plants, the locations of which are indicated by blank spaces in table 1, developed crown-gall. No record was made for these plants.

The experiment was terminated on June 1, 1920. At this time, after removing the plants from the soil, tissue plantings from the crown of each plant were made in sterilized potato-agar with the idea of determining whether or not the fungus was present in the tissues. In making these cultures the following procedure was employed: The crown-segments of the plants were first washed thoroughly by holding them in running water under the tap and scrubbing with a stiff brush. The outer bark was then scraped away and the segment immersed in 50 per cent alcohol for three minutes, and then in 1–1000 mercuric chloride solution for ten minutes. Following this the segment was rinsed in 95 per cent alcohol which in turn was removed by flaming. Bits of tissue were then removed with a flamed scalpel and placed in sterilized potato-agar in previously poured Petri dishes. Eight plantings were made from each plant.

TABLE 2

Record of the number of rose plants and the total and average number of blossoms produced by plants growing in infested and uninfested soil during the second and third seasons. The starred letters indicate sections containing infested soil

SECTION	NUMBER OF PLANTS	TOTAL BLOSSOMS		AVERAGE BLOSSOMS PER PLANT	
		1918–1919	1919–1920	1918–1919	1919–1920
A*	16	586	303	36.62	18.94
B*	16	510	379	31.87	23.69
C*	16	465	383	29.06	23.94
D*	20	586	429	29.30	21.45
E	20	760	532	38.00	26.60
F	25	945	805	37.80	32.20
G	18	686	621	38.11	34.50
H	20	803	636	40.15	31.80
I	19	820	673	43.16	35.42
J*	16	550	363	34.37	22.69
K*	16	519	372	32.44	23.25
L*	16	613	361	38.31	22.56
All Sections	218	7843	5857	35.98	26.87

The plants from which *C. scoparium* was isolated are indicated in table 1. Severe measures to insure thorough surface sterilization were made necessary by the heavy infestation of the soil; and since the fungus frequently does not penetrate deeply into the tissues—in many cases probably not beyond the bark—too much emphasis should not be placed upon successful isolations as criteria of disease. It is obvious, however, that the results obtained warrant a certain amount of confidence in their value. Successful isolations of the fungus were obtained from 77 of 116 plants growing in infested soil, and from but 4 of 102 plants growing in supposedly uninfested soil. These results, especially when correlated with the production of blossoms (see page 132), are significant in indicating the high percentage (66.38 per cent) of infected plants from infested soil, as well as indicating no small amount of accuracy relative to the isolation methods used. No certainty exists, considering the strenuous treatment given to insure surface sterilization, that the fungus was isolated from every diseased plant. Consideration also should be given to the possibility of the fungus having died after penetrating and killing certain tissues of the host. Plants referred to in the following paragraphs as being diseased are those from which successful isolations of the fungus were made.

Cankers due to *C. scoparium* other than those formed at the crown of the plants were relatively uncommon in the house throughout the duration of the experiment. Occasionally the stubs left in cutting the blossoms were attacked by the fungus, but in no great number of instances did the pathogene work into the stem to any appreciable distance. These results are contrary to the statements of several growers, who claim that the fungus is rapidly and easily disseminated to parts of the plant above the soil resulting in material quantities of dead shoots. That the above-ground parts of plants were attacked in so few instances together with the fact that only four of the plants in the presumably uninfested soil were attacked at the crown, may be due to the existing sanitary conditions of culture. Great care was exercised by the gardener in preventing the mixing of the soil in the various sections and the accumulation of dead plant-parts.

The growth of grafted plants in solid beds is not conducive to the production of shoots from parts beneath the soil. In the few instances, however, where such shoots were formed by plants growing in infested soil, these shoots were early attacked and killed by the fungus. On the other hand such shoots formed by plants in uninfested soil remained healthy.

DISCUSSION OF RESULTS

As stated above, the records for 1918–1919 consisted of the number of blossoms cut from each section. These are given in table 2. From 116 plants growing in infested soil, a total of 3829 blossoms, or an average of 33.01 per plant were obtained, whereas 102 plants in uninfested soil produced 4014 blossoms, or an average of 39.35 per plant. This difference of over six blossoms per plant would determine in a large measure whether or not such plants could be grown at a profit (see page 133). As to whether or not the chemicals added to the soil were of any influence during the second season, or were instrumental in affecting the yields is problematical. As pointed out by Muncie[4] applications of potassium sulphate and of lime decrease, and of acid phosphate increase the yield. It should be noted that the plants in section D to which acid phosphate was added gave an average of 29.30 blossoms, while those in section B to which potassium sulphate was added, and in section L to which lime was added, gave an average of 31.87 and 38.31 blossoms, respectively. The variety Ophelia may differ from the varieties Richmond, Killarney and Bride, as used by Muncie, in its response to the chemicals in question; or possibly the conditions under which Muncie worked were not comparable to those of the experiment of the writer. Unless these differences exist there is evidence that no effect was exerted by chemicals during the period covered by these records. It would seem that the factor directly responsible for this reduction is that of disease.

Doubtless more importance is to be attached to the results for the season of 1919–1920, during which the record of the production of blossoms by individual plants was taken. The positions of the plants in the beds, and the number of blossoms produced are indicated in tables 1 and 3. In table 2 a comparison by section is made of the yields of 1918–1919 and 1919–1920. During this third season a total of 5857 blossoms were cut from the 218 plants, of which 4117 were obtained from 137 healthy plants and 1740 from the 81 plants from which the fungus was successfully isolated. The healthy plants thus averaged 30.05 blossoms, and the diseased, 21.48, per plant.

Since, as stated above, the success or failure of isolation of the fungus from plants may have been of doubtful value as a means of determining whether or not a plant was diseased, it seems advisable to compare the yields of the plants growing in uninfested soil with those growing in infested soil. The 116 plants growing in infested soil produced 2590

[4] Muncie, F. W. The use of commercial fertilizers in growing roses. Illinois Agric. Exp. Sta. Bull. 196: 510–564, *2 fig.* 1917.

blossoms, or an average of 22.33 per plant; the 102 plants growing in uninfested soil yielded 3267 blossoms, or an average of 32.03 per plant. These results are probably the most striking of any obtained, the reduction of blossoms being obviously due to the single factor of infested soil. This difference, as indicated by sections, may be seen in table 3. Thirty-nine supposedly healthy plants in infested soil produced 925, or an average of 23.72 blossoms per plant; 77 supposedly diseased plants in infested soil gave 1665, or an average of 21.62 blossoms per plant. Thus there is strong evidence that the supposedly healthy plants were affected by some factor, the most obvious one being the presence of the fungus in the soil. This evidence is further strengthened by the fact that 98 healthy plants growing in the presumably uninfested soil produced 3192 blossoms, an average of 32.57 per plant, whereas the 4 diseased plants in this soil yielded 75 blossoms or an average of 18.75 per plant. The reduction of about 10 blossoms per plant which must be attributed to the presence of the fungus in the soil, with the resulting infection of the plants, is more than sufficient to determine whether or not diseased plants can be grown at a profit.

TABLE 3

Record of the number of diseased and healthy rose plants showing the total and average number of blossoms produced during the third season. Starred letters indicate sections containing infested soil

SECTION	NUMBER OF PLANTS	NUMBER OF FLOWERS	NUMBER OF DISEASED PLANTS	NUMBER OF FLOWERS		AVERAGE NO. OF FLOWERS	
				DISEASED PLANTS	HEALTHY PLANTS	DISEASED PLANTS	HEALTHY PLANTS
A*	16	303	13	259	44	19.92	14.67
B*	16	379	8	170	209	21.25	26.12
C*	16	383	9	215	168	23.89	24.00
D*	20	429	15	327	102	21.80	20.40
E	20	532	2	49	483	24.50	26.83
F	25	805	2	26	779	13.00	33.87
G	18	621	..	...	621	....	34.50
H	20	636	..	...	636	....	31.80
I	19	673	..	...	673	....	35.42
J*	16	363	11	216	147	19.64	29.40
K*	16	372	12	291	81	24.25	20.25
L*	16	361	9	187	174	20.79	24.86
All sections	218	5857	81	1740	4117	21.48	30.05

Reference to table 2 will show that in all cases fewer blossoms were produced by the plants in each section during 1919–1920 than during 1918–1919. Undoubtedly part of this reduction was due to the aging of the plants. The average decrease per plant for those growing in infested soil was 10.68, while for those growing in uninfested soil the average decrease was 7.32 blossoms per plant. The decrease in the number of blossoms from the plants in uninfested soil is significant as indicating the normal reduction in yields of plants carried into the third year, while 3.36 blossoms per plant represents the decrease over and above the normal decrease, due to the parasite. This slight increase over the normal reduction may be taken as evidence that the fungus had not advanced much further into the tissues during the last year that the plants were carried.

Few figures are available from which to determine the cost of the production of blossoms or to indicate the margin of profit on which commercial growers carry their plants. Trade journals in which the prices of blossoms are given show that the returns to the grower from Ophelia roses vary from two to sixteen cents each, with even higher values at certain times of the year. A fair average value per blossom to the grower is about ten cents. On this basis, a reduction of ten blossoms per plant, which as shown above, must be ascribed to the influence of the parasite, would mean a loss of one dollar per plant per season, for plants growing in soil infested with the fungus. Obviously this loss must be deducted entirely from the profits of the grower, for the cost of growing diseased plants would be the same as that for healthy plants. Consideration must also be given to the failure of diseased plants to respond to forcing during periods when there is the greatest demand for blossoms, and consequently the highest returns to the grower. Although no figures are at hand it is probable that blossoms from diseased plants are of inferior quality, in that the stems are shorter. Certainly the average grower does not sell blossoms at a sufficiently large margin to enable him to realize a profit on plants which are producing ten less than the normal number of blossoms.

Although the number of plants used in the experiment is not large, it is felt that the results amply justify all of the conclusions that are drawn from them. The house was small and the heating system so constructed and arranged that definite and even temperatures could be maintained throughout. Since the heating pipes were next to the two outside beds it is probable that the temperature of the soil and that of the air surrounding the plants in these beds was slightly higher than corresponding temperatures of the middle bed. However, the plants in each section of the two outside beds were subjected to the same con-

ditions, and since one section was a check, any differences due to uneven temperatures are evident from the data presented. The soil was thoroughly mixed before being placed in the beds, to ensure uniformity, thus eliminating the factor of soil variation. A method of maintaining a constant moisture content of the soil would possibly have helped to eliminate this varying factor, but it is doubtful whether or not the slight variation which occurred in the sections was sufficient to influence the results.

SUMMARY

1. The data here presented were obtained from an experiment conducted throughout a period of three years. Ophelia roses were grown under glass in infested and uninfested soil, and a record taken of the number of blossoms produced under these conditions. The losses caused by *Cylindrocladium scoparium* Morgan were measured by the reduction in the number of blossoms.

2. Losses are to be measured by the decrease in yields of blossoms and not by the death of the host.

3. Calcium sulphate, ammonium sulphate, potassium sulphate, acid phosphate and lime under the conditions of the experiment exerted no influence on the losses caused by the parasite.

4. A decrease of about 10 blossoms per plant represents the annual loss when plants are grown in infested soil. This reduction means a loss to the grower of at least one dollar per plant.

5. Mildew and black-spot were readily controlled by three or four applications of sulphur-lead dust, made at weekly periods, combined with judicious regulation of water and temperature.

CORNELL UNIVERSITY
ITHACA, NEW YORK

A LOTUS LEAF-SPOT CAUSED BY ALTERNARIA NELUMBII SP. NOV.

ELLA M. A. ENLOWS, AND FREDERICK V. RAND.

WITH PLATE IV AND ONE FIGURE IN THE TEXT

This leaf-spot disease was first observed by Rand in the summer of 1913 at Kenilworth, D. C., and in autumn of the same year at the New York Botanical Gardens on leaves of Egyptian lotus (*Nelumbium speciosum*). From the general character of the disease it was thought to be of parasitic origin, and in the summer of 1914 the authors began a joint investigation of the cause.

Since 1913 the disease has been seen by both of us at Riverton, N. J., again at the New York Botanical Garden and Kenilworth, D. C., and by Rand at Arlington, N. J., and Pittsburgh, Pa.

DESCRIPTION OF THE DISEASE

The disease begins as very small, smooth, reddish brown flecks, which increase in size until 5 or 10 mm. in diameter. They may be roundish or somewhat irregular in form with a tendency toward the formation of concentric bands of alternately lighter and darker brownish tissue (Plate IV A). The margin is definite. In some cases the spots are surrounded by a lighter green halo. When the spots are very numerous many of them coalesce, and the leaf gradually dries and curls from the margin inward.

ISOLATION OF THE FUNGUS

On July 22, 1914, diseased material was collected at Arlington, N. J., and the Brooklyn Botanical Garden, New York City. Free-hand sections from these leaves showed the presence of brownish septate mycelium and plates were poured in the usual manner, using cornmeal agar. The majority of the colonies coming up on these plates consisted of but one type of fungus (Isolations R214 and R218). The mycelium was brownish, septate and similar to that observed in the free-hand sections of the diseased leaves. In less than a week abundant spores of the Alternaria type had developed on these colonies. During the years 1914 and 1918, inclusive, 23 isolations were made by both authors from material from the Brooklyn Botanical Garden, New York City, Arlington and Riverton, N. J., Kenilworth, D. C., and from artificially inoculated plants growing in the greenhouse. The same type of fungus was obtained in all these isolations, and all were proved to be infectious by inoculation into healthy plants from pure cultures.

INOCULATIONS

The first inoculations were made on August 8, 1914, in the following manner (Isolations R214 and R218): Leaves of lotus with petioles about 5 cm. in length were floated in water contained in large, cylindrical glass dishes, provided with covers. Moistened conidia were placed on the upper leaf surfaces, some of them being pricked in with a sterile needle, and others being merely laid on the uninjured surface. The covers were allowed to remain on the dishes for 5 days. At the end of 7 days typical spots had appeared, varying in size from tiny flecks to 5 mm. in diameter. The infections occurred not only at the pricked points, but also from the conidia placed on uninjured leaf tissue. Six leaves were used for each isolation in this experiment, and two uninoculated leaves were kept under the same conditions as controls. The controls remained healthy throughout the experiment (about 2 weeks). Reisolations (En 248, En 251) were made from 2 leaves inoculated with *R 214* with which successful infections were obtained. In a similar manner all of the 23 isolations of the fungus were tested, using young, healthy lotus leaves, always keeping several uninoculated leaves under the same conditions as controls. Most of the leaves inoculated from each isolation showed within 5 to 7 days typical reddish brown spots, while in each experiment the controls remained healthy.

In addition to these laboratory inoculations to cut lotus leaves, lotus plants growing in large tubs of soil and water in the greenhouse and out of doors were also inoculated. The method of inoculation was similar except that moist air conditions were obtained by placing bell jars on supports so that the base of the bell jars dipped just below the surface of the water (Plate IV B). The bell jars were left over both the inoculated and control leaves for 3 to 5 days. Fourteen of the different isolations of the fungus were used for these greenhouse inoculation experiments and successful infections were obtained with all (Plate IV A, C). Infections occurred in a majority of cases only where the conidia were introduced, but in one or two instances the infection later spread from the inoculated to the contiguous uninoculated leaves. The controls remained healthy except in these cases. Reisolations were made from all of these artificially infected plants with which the disease was again reproduced. Four of the most virulent of the reisolations were carried successively through healthy plants in order to observe the effect on the virulence of the organism by successive passage through its host. But little difference in virulence could be detected in those reisolations which had been passed four or five times through the host and the original isolations from which they had been subcultured.

The results of the inoculations conducted on lotus plants growing under normal conditions in the greenhouse and out of doors, together with the laboratory inoculations described, leave no doubt as to the pathogenicity of this species of Alternaria to lotus.

Observing the morphological similarity of this species of Alternaria to *Alternaria brassicae nigrescens* (causing leaf-spot of cantaloupe) upon which the authors were also working, several cross-inoculation experiments were carried out with both species of fungus to cantaloupe and lotus. No cross-infections were obtained.

SOME CULTURAL CHARACTERS

It was found that for growth and sporulation corn-meal agar furnished an excellent medium. Consequently this medium was used for most of the cultural work. On corn-meal agar slants growth is evident after 24 hours as a thin fluffy white surface layer, which at the end of 48 hours becomes tinged with green, and shortly the entire surface is dark green to black, the color also extending down into the medium, where the hyphae have penetrated. At the end of 14 days the thin surface layer is pale smoke gray[1] to smoke gray, and below this the color is olivaceous black to black. The time elapsing before spore-formation varies according to the temperature and perhaps also according to the moisture content of the medium. In some cases spores begin forming at the end of 48 hours and in others not before 3 to 5 days. On potato, growth is very heavy and spores are produced fairly promptly. For the first 5 days the color is lighter than the growth on corn-meal agar, but after ten days it is dark olivaceous to black. A great many flask cultures were made using corn-meal plus litmus milk. In these flasks there is a dense black surface growth, not extending downward. Directly beneath the surface growth there is a layer of brownish pink pseudoparenchyma. The medium is bleached to a cream color throughout, while the control flasks are a dark, livid purple.

On beef agar the mycelium develops slowly, the conidia are produced sparingly and are usually distorted. On this medium the thin surface layer is vinaceous buff, below which the color is brownish.

To determine the relation of temperature to growth corn-meal agar cultures were placed in a large ice thermostat provided with ten compartments ranging in temperature from 2° C. to 18° C. Below 5° C. growth was extremely slow. At the end of 10 days there was no growth at 2° C., but at the end of two weeks in the compartment the colonies

[1]Ridgway, Robert. Color Standard and Color Nomenclature. *43 p. 53 col. pl.* Washington, D. C., 1912.

were 2 to 3 mm. in diameter. At 5° C. growth was slightly more rapid, but no spores developed at either of these low temperatures, even after one month's incubation. At 9° C. growth entirely covered the slant at the end of 10 days, but no spores had at this time developed below 15° C. At the end of one month growth had covered the surface of the slants with production of spores in compartments ranging from 8° C. to 18° C.

The fungus does not lose its virulence readily when grown on a suitable medium at room temperature. Isolations carried in the laboratory for several years and transferred at intervals of 1 to 3 months have always given infections when young cultures were inoculated into healthy lotus plants.

For the purpose of determining the longevity of this fungus cultures were made on corn-meal agar and placed in storage cupboards at room temperature. These cultures were tested for viability at varying intervals up to two years, and even though at this time the agar slants had dried down to hard balls in the bottom of the tubes, nevertheless scrapings from these balls were found to contain conidia of *Alternaria nelumbii* which grew with moderate promptness when transferred to fresh media. Inoculations were later made from these transfers and infections obtained.

MORPHOLOGY AND TAXONOMY

The mycelium is dark-colored, frequently olivaceous. The conidiophores are slightly lighter in color, 1- to 4-septate, usually erect, rarely in clusters on the host or in media. The conidia are oblong or obclavate to globose, olivaceous, measuring 8 to 18μ wide by 14 to 65μ long, average $14 \times 35.2\mu$, with a varying number of oblique, transverse and longitudinal septa (figure 1) where slight constriction occurs, terminating usually in a short, 1- to 2-septate beak, which is hyaline. Round to globose one-celled conidia are frequently found. The conidia have been found in chains of 3 to 8. Their irregular form and size is especially noticeable on media not well adapted to the growth of the

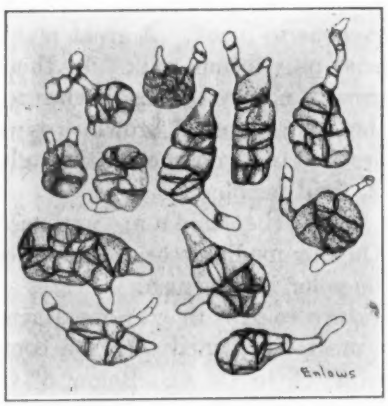


FIG. 1. GERMINATING CONIDIA OF ALTERNARIA NELUMBII

Drawn from a 48-hour corn meal agar culture (Enlows).

fungus. No perfect stage has been discovered for the fungus, but the fact that the conidia are able to germinate after remaining dry in the laboratory for so long a period as 2 years certainly would indicate that the conidia are probably carried over winter on dead leaves and other debris. It grows well saprophytically, so it is also possible that it might growing during the warmer periods of the winter on fallen leaves.

The fungus does not spread far beyond the diseased area on the lotus leaf. Numerous paraffin sections of affected leaves were made, various stains used and careful studies made in an effort to determine the manner of entrance, but in no case were any hyphae found entering stomata. The fungus may either take advantage of wounds which may be present on the leaf surface, or effect entrance through the dissolving effect of toxic products resulting from its growth. The latter seems probable in view of the fact that sections made from inoculated material fixed very soon after the spores had started germinating showed complete disintegration of the epidermal cells immediately underlying the germinating conidia, and disorganization of the palisade cells directly beneath. Older spots show complete disorganization of all the tissue systems in the vicinity of the fungus, but not beyond it. Cells immediately adjacent to those whose cytoplasm has degenerated into more or less homogeneous coagulation products may still contain normally-staining nuclei and chloroplasts.

The fungus as described above differs from any other so far described on Egyptian lotus, and belongs undoubtedly in the genus Alternaria. The name *Alternaria nelumbii* sp. nov. is therefore suggested.

SUMMARY

The leaf-disease of Egyptian lotus (*Nelumbium speciosum*), here described as caused by *Alternaria nelumbii*, appears as very small, smooth, reddish brown flecks, which later increase in size until they reach a diameter of 5 to 10 mm. The tendency toward the formation of concentric rings is characteristic. The pathogenicity of *Alternaria nelumbii* to lotus has been demonstrated by repeated isolation and successful reinoculation into healthy plants. No perfect stage was discovered, but the longevity of the fungus under laboratory conditions renders it probable that under field conditions it may also be capable of living over long periods of time in the conidial stage.

LABORATORY OF PLANT PATHOLOGY
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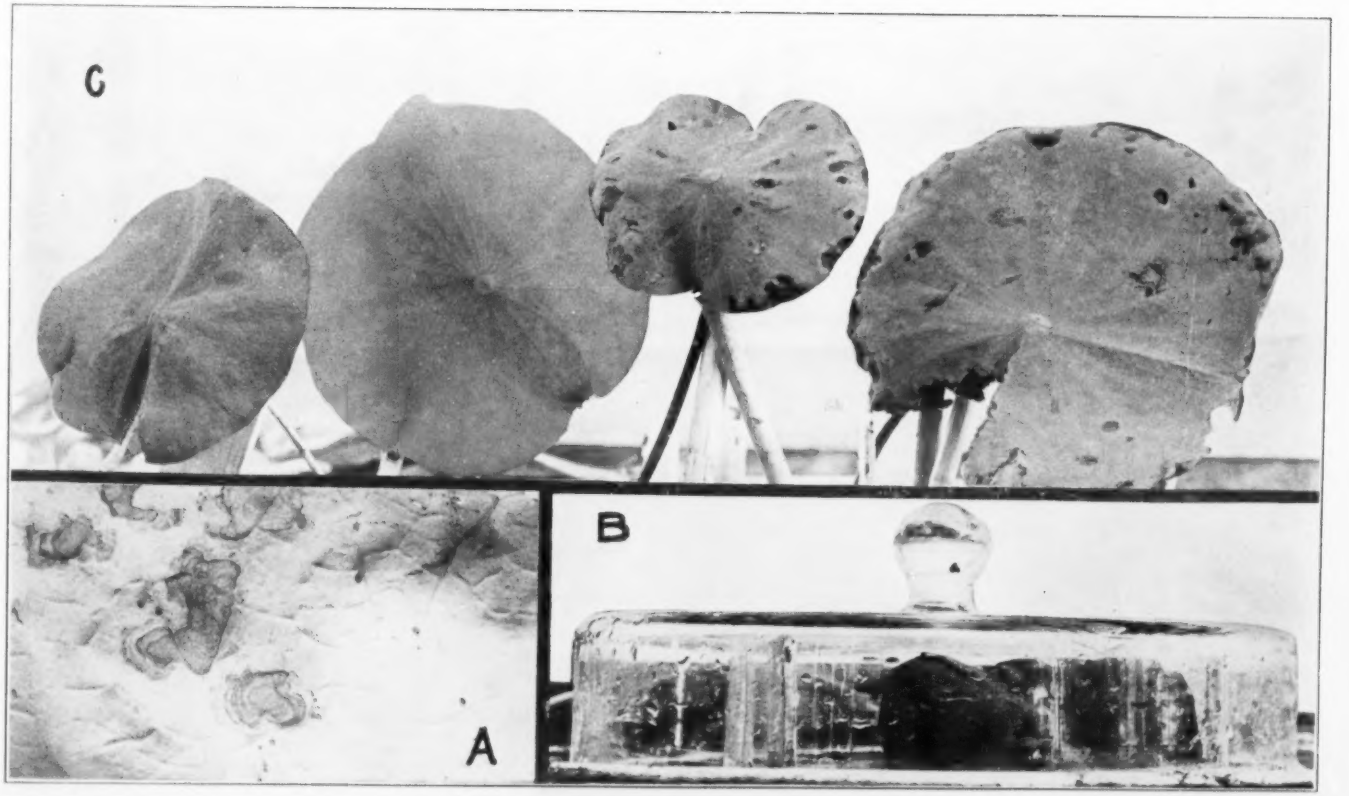
PLATE IV. ALTERNARIA NELUMBII ON LOTUS.

Fig. A. Typical leaf-spots from inoculations of July, 1915. ×2.

Fig. B. Method of forming damp chamber for inoculation of lotus leaves.

Fig. C. Lotus leaves to right inoculated December 21, 1914, with spores of *Alternaria nelumbii* (246) from cornmeal agar culture 6 days old. Two leaves to the left are controls treated in the same way but not inoculated. Photographs by J. F. Brewer.



ALTERNARIA NELUMBII ON LOTUS

THELEPHORA TERRESTRIS, T. FIMBRIATA, AND T. CARYOPHYLLEA ON FOREST TREE SEEDLINGS

JAMES R. WEIR

WITH PLATE V.

During the field seasons of 1912–1916 a number of specimens of the genus Thelephora were sent to the writer by field and nursery men of the several National Forests in the Northwest with inquiry as to their pathological significance. The specimens were usually accompanied by some such statement as "growing around the base of young trees," "growing around and over seedlings," or "on roots of trees." Two of the species sent have not previously been reported as causing injury to forest tree seedlings.

THELEPHORA TERRESTRIS (EHRH.) FR.

Robert Hartig (4, 5) reported *Thelephora terrestris* Ehrh. (*T. laciniata* Pers.) as sometimes occurring so abundantly in silver fir, spruce and Weymouth pine plantations that young plants were frequently completely enveloped. This strangling process continued until the tip of the seedling barely projected above the surface of the fungus, resulting in death. In like manner, Hartig reports the smothering of beech seedlings when growing close together. The destruction of spruce seedlings or the branches of older trees which may lie on the ground, by *Thelephora terrestris*, is mentioned by Frank (2, p. 235). The Weymouth pine, firs and red beech were seldom affected. Tubeuf (9, 10) gives brief accounts of the injury to young fir trees and of one-year-old beech seedlings by *Thelephora terrestris*.

An anonymous account (8) of the injurious effects of this fungus on various seedlings, in England, shows that it may extend up the stem for a distance of a foot or more.

Mention of the injury sometimes resulting from the growth of *Thelephora terrestris* around tree seedlings is made by Lindberg (6).

Freeman (3) refers to the fungus in relation to forest tree seedlings, but does not think much damage may result from it in Minnesota. Later references may be found in text-books on plant diseases by Sorauer, Stevens, and others.

It will be seen from the foregoing that *Thelephora terrestris* is the only member of the genus so far reported as injurious to forest tree seedlings. Schrenk (7) has described a root rot of apple trees caused by *Thelephora galactina* Fr. (Nov. Symb. Myc., pp. 136) but this fungus is now classed by Burt in Corticium. Schrenk states that the rot caused

by this organism was transferred from oak roots to young apple trees, killing the latter within a year.

The states and localities in which *Thelephora terrestris* has been observed to injure forest tree seedlings are recorded as follows:

British Columbia: encrusting the stems of *Acer macrophyllum*, Salmon Arm: *Pseudotsuga taxifolia* seedling partially enveloped, Agassiz.

Washington: on two-year *Pseudotsuga taxifolia* seedling near Olympia; completely enfolding the stem and needles of two-year seedlings of *Pinus monticola*, Metaline Falls, matting the leaves of a clump of two-year seedlings of *Thuja plicata*, Newport.

Oregon: growing around the base of young seedlings of *Pinus ponderosa*, Austin.

Idaho: growing around the stem and partially enveloping the needles of *Abies grandis*, Priest River; around stems of young *Pseudotsuga taxifolia*, *Pinus ponderosa* and *Picea engelmani* on nursery area at Priest River Experiment Station.

Montana: enveloping the base of an eight-year-old *Larix occidentalis*, not connected with the forest soil, Pattee Canyon, Missoula; (Fig. A) partially enveloping a young seedling of *Pinus contorta*, Anaconda, and on various pine seedlings at the Forest Service Nursery, Haugan.

Utah: growing around stem of young *Pinus ponderosa*, locality not given (Forest Service).

Wyoming: enveloping seedlings of *Pinus contorta*, Encampment.

South Dakota: attached to stem and roots of young *Acer negundo*, Camp Crook.

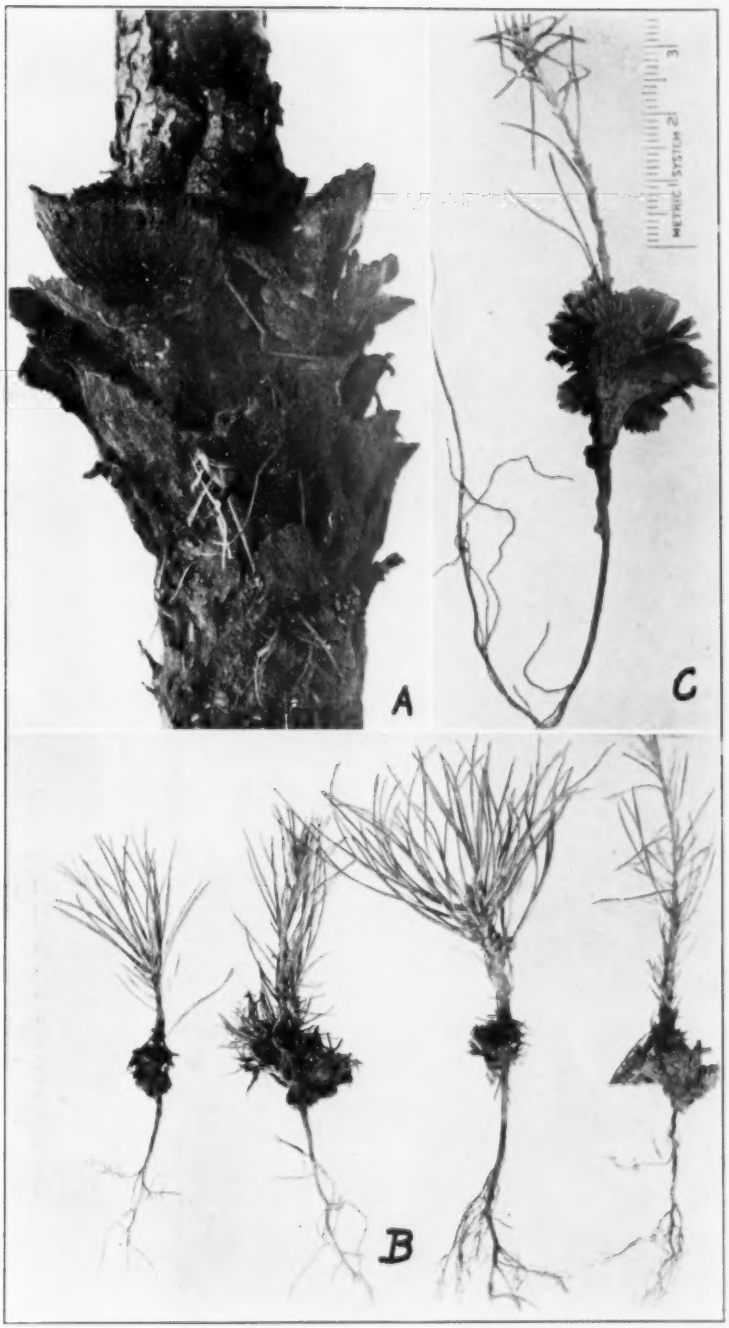
Minnesota: enveloping the needles of *Picea mariana*, Ely.

Michigan: growing at base and attached to the epidermis of seedlings, *Pinus divaricata*, East Tawas.

Indiana: surrounding the stem of two-year-old *Quercus alba*, Weirtown.

Various plants other than forest tree seedlings are overrun and killed by this fungus. An interesting case of the destruction of a seedling of *Ribes prostratum* is shown by a collection made by Dr. A. S. Rhoads, at North Conway, New Hampshire. *Thelephora intybacea* (Pers.) Fries, which resembles *T. terrestris*, is observed to strangle various plants, but has not been recorded on forest tree seedlings.

The fact that *Thelephora terrestris* may envelop a young seedling much more rapidly and more permanently than a number of the fleshy and coriaceous Polypores, is the chief reason why attention is given it, and especially because the fungus frequently appears in the sandy soil of forest tree nurseries. The fungus usually occurs where the seedlings are dense enough to shade the ground. Although the behavior of the fungus is often apparently parasitic, repeated examination of the



THELEPHORA SPP. SMOTHERING CONIFER SEEDLINGS.

young pine seedlings enveloped by it has not shown any living part to be invaded. The mycelium ramifies in the surface soil and merely penetrates the dead outer tissues of any plant encountered. If the plant is completely smothered by the fungus, its mycelium may penetrate into the tissues of the leaves through the stomata and even form a brown layer between the dead bark and wood of the stem. The fungus is very often found hibernating in the thick moist bark at the base of young trees, in which case the mycelia may not be connected with the forest soil (Fig. A). The fungus may draw its nourishment entirely from a moist woody substratum. Burt (1, p. 219, 220) points out the interesting fact that the substratum from which the fungus draws its nourishment exercises an influence on the form and color of the fructification.

When not attached to any woody material the fruiting body is a dark fuscous color and consists of a flattened cluster of small imbricated pileoli, or is composed of a series of super-imposed pileoli. On wood the fructification is of a redder color and reflexed. The habit of growing on both humus and decaying wood is exhibited by a number of the higher fungi which the writer has known to smother seedlings by growing around them. Among them may be mentioned *Polyporus tomentosus*, *Polyporus perennis*, and *Clavaria stricta*.

During damp weather the fungus grows very rapidly and may envelop a small seedling in a comparatively short time. A one-year-old seedling of *Thuja plicata* 5 cm. high, having at its base a young fungus, was completely enveloped in 7 days. A rule set up by the side of the seedling showed that the fungus grew more rapidly during the night, registering in one instance an extension in height of 6 mm. in one night.

Seedlings in nurseries affected with *Thelephora terrestris* should be pulled up and burned. It was found that when this was done at the Savanac Nursery the fungus did not develop in the beds the following year. The use of duff or forest soil in the seed beds promotes the growth of the fungus.

THELEPHORA FIMBRIATA SCHW.

In the seed beds of the Forest Service nursery at Haugan, Montana, during July, 1914, and again in 1915, *Thelephora fimbriata* appeared in considerable quantity. Two- and three-year-old seedlings of *Pinus ponderosa* were in some cases completely enveloped. The usual type of injury was the complete envelopment of the stem and matting of the lower needles (Fig. B). The late E. C. Rogers reported to the writer that in 1915, at the Haugan Nursery, he found 18 yellow pine seedlings in one bed affected by this fungus.

Seedlings of *Pinus rigida* partially overgrown with *Thelephora fimbriata* were received from H. H. Morgan, of the Pennsylvania Department of Forestry in 1916. The seedlings were from the State Nursery on the Black Forest in Lycoming County. The fungus was reported common.

THELEPHORA CARYOPHYLLEA (SCHAEFFER) FR.

This fungus was observed growing up around one-year-old seedlings of *Larix occidentalis* (Fig. C) and *Tsuga heterophylla* in seed beds at the Priest River Experiment Station, Idaho, in 1915. Since then it has not reappeared, but is abundant in the surrounding forest, where it is occasionally found enveloping the stems of young lodgepole pine seedlings. This species is one of the most beautiful of the genus and is frequently very abundant in damp coniferous woods.

SUMMARY

An account of the occurrence of *Thelephora terrestris* on coniferous seedlings in nurseries and on other plants is given. The injury of coniferous seedlings by *Thelephora fimbriata* and *T. caryophyllea* is reported for the first time.

LABORATORY OF FOREST PATHOLOGY
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SPOKANE, WASHINGTON

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BRIEFER ARTICLES

MOTION PICTURES OF ZOOSPORE PRODUCTION IN PHYTOPHTHORA

G. H. Godfrey and R. B. Harvey

With Plate VI

The liberation of free swimming spores from the zoosporangia of certain of the Phycomycetes has been a process of interest to all pathologists. Numerous investigators have watched and described the events which occur. All have no doubt wished that they might trace more closely the rapidly occurring phenomena step by step, and that they might record them graphically for showing to students or others to whom it is often difficult to give an actual demonstration. It has been the pleasure of the authors to record the sequence of events in a species of Phytophthora by means of the motion picture camera. The film is now available for projection before students, and may be secured by application to the United States Department of Agriculture.

The material used was a species of Phytophthora discovered by Godfrey on rhubarb and as yet unpublished. The process of zoospore formation is remarkable for the rapidity with which it occurs. One sees first as in figure 1 of plate VI,[1] the normal granular protoplasm previous to the appearance of cleavage lines which indicate the approaching activity. In figure 2 practically the same stage is shown. Here a good view of the apical papilla is given under the oil immersion lense. The inner line of the wall of the papilla is still visible, but the tip seems to have softened and swelled up somewhat. In figure 3 this inner line has disappeared and the tip is much swollen. The protoplasm has rounded up into zoospores. This is the last picture taken before the papilla gives way to the increasing pressure within the sporangium. The pictures were taken at the rate of sixteen per second, which is fast enough to show that the tip is being gradually burst outward. Figure 4 was taken as the zoospores were emerging. Some are still within the sporangium while those outside have immediately become active and are caught by the camera swimming about in the field. Figure 5 shows the last zoospore emerging from a sporangium. The movements in passing through the pore are amoeboid, for the opening is much smaller than the zoospore. Several other interesting processes in the development of this fungus have also been recorded by the camera.

[1] Grateful acknowledgement is made of credit due Mr. James F. Brewer of the Office of Plant Pathology for enlargements from the motion picture film used to illustrate this article.

Similar projects with other organisms are under consideration and it is hoped that they may result in the more general application of cinematophotomicrography to teaching and extension work in the biological sciences.

BUREAU OF PLANT INDUSTRY

A MOSAIC OF SWEET AND RED CLOVERS

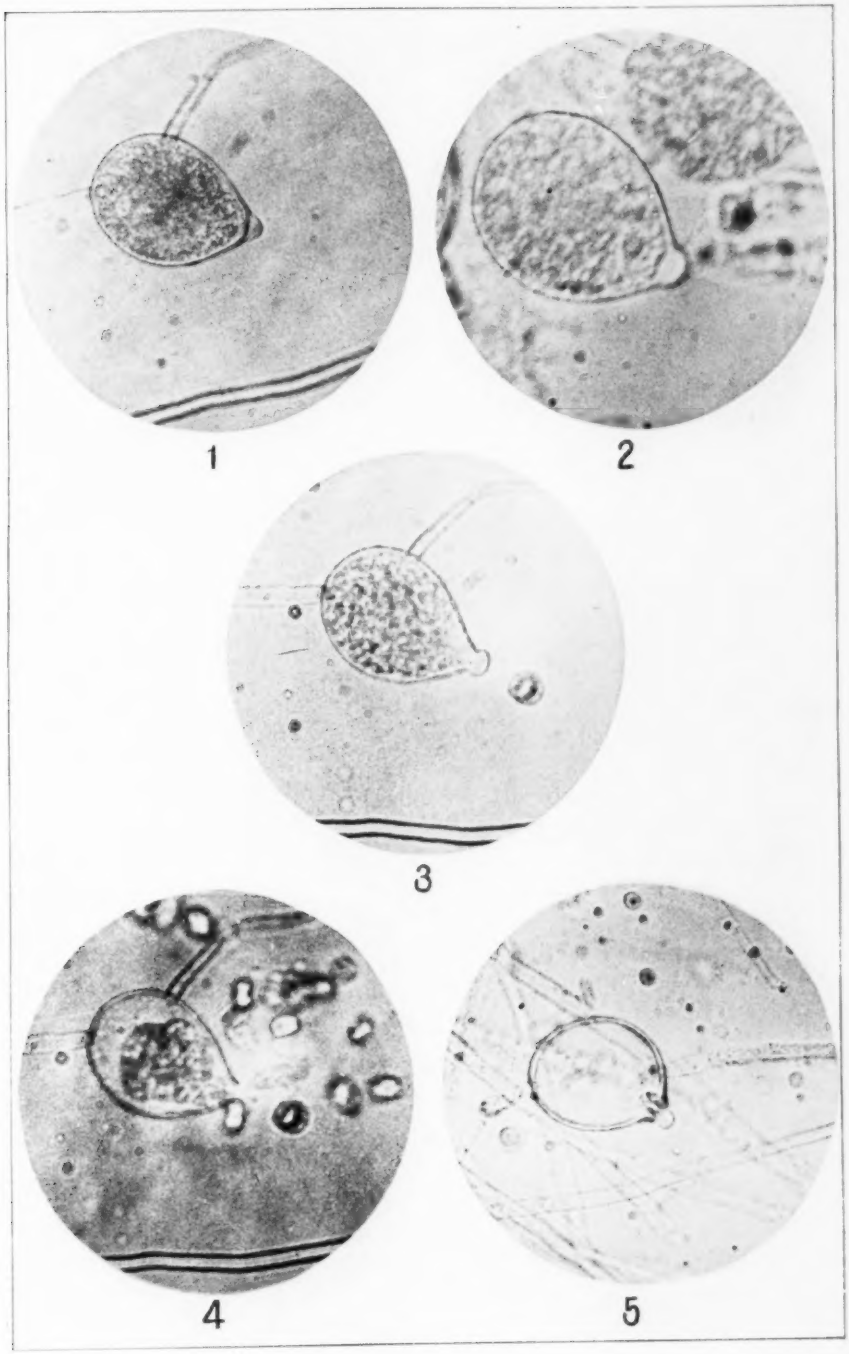
JOHN A. ELLIOTT

WITH ONE FIGURE IN THE TEXT

In the November, 1920, issue of Phytopathology, H. R. McLarty (2) published a short note on a mosaic of sweet clover (*Melilotus alba*) which he has had under observation. Rosen (4) had previously mentioned this disease as being under observation at the Arkansas Experiment Station, in his bulletin on The Mosaic Disease of Sweet Potatoes published in April, 1920. Clinton (1) also mentions a chlorosis of common species of clover in his publication on Chlorosis of Plants published in 1915.

The mosaic of sweet clover first came to my attention in the summer of 1917 when it appeared on about fifty per cent of the sweet clover plants on the University of Arkansas campus. As the plants were growing wild and were rather widely scattered, inoculation experiments were attempted on several apparently healthy plants, using crushed leaves from diseased plants as the inoculum. The results in all cases were positive. Several mosaic plants were marked for observation on the following season. All of the marked plants which grew the following year showed the characteristic symptoms of the disease early in the season.

In September of 1919 it was noted that not only the sweet clover but the red clover (*Trifolium pratense*) showed very marked symptoms of the disease. At this time of the year fully seventy-five per cent of the red clover plants on the campus were very severely attacked, the symptoms being much more pronounced on the red clover than on the sweet clover. Several healthy red clover and sweet clover plants were placed in pots in the greenhouse and after a suitable period of observation for signs of the disease due to natural infection, cross inoculations with infectious material from diseased plants were attempted. All attempted inoculations were successful and there appeared to be no difference in the disease from the different sources. Inoculations were also attempted on white clover (*Trifolium repens*) and alfalfa (*Medicago sativa*), none of which were successful. White clover and alfalfa were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growing intermixed with the infected sweet clover and red clover everywhere on the campus, but no appearance of the mosaic symptoms on either of these plants has been noted in the four summers' observations, and repeated attempted inoculations have failed.

To have a more satisfactory check on the work that had been done with transplanted plants, seed of several legumes, from commercial seed houses, was secured and planted in pots in the greenhouse. The legumes chosen[1] were horse bean (*Vicia faba*), alfalfa, white clover, sweet clover, spotted burr clover (*Medicago arabica*), and red clover. When these plants had attained sufficient size for convenient handling and were seen to be free from any symptoms of mosaic, two plants of each species were inoculated from each of the sources of infection, *i. e.*, diseased sweet clover and red clover plants. Inoculations from both sources appeared equally virulent on the different plants proving susceptible. The disease usually appeared simultaneously on all of the plants inoculated in from ten to fifteen days. The plants which proved susceptible were sweet clover, red clover, burr clover and horse bean. No infections occurred on white clover or alfalfa even after repeated

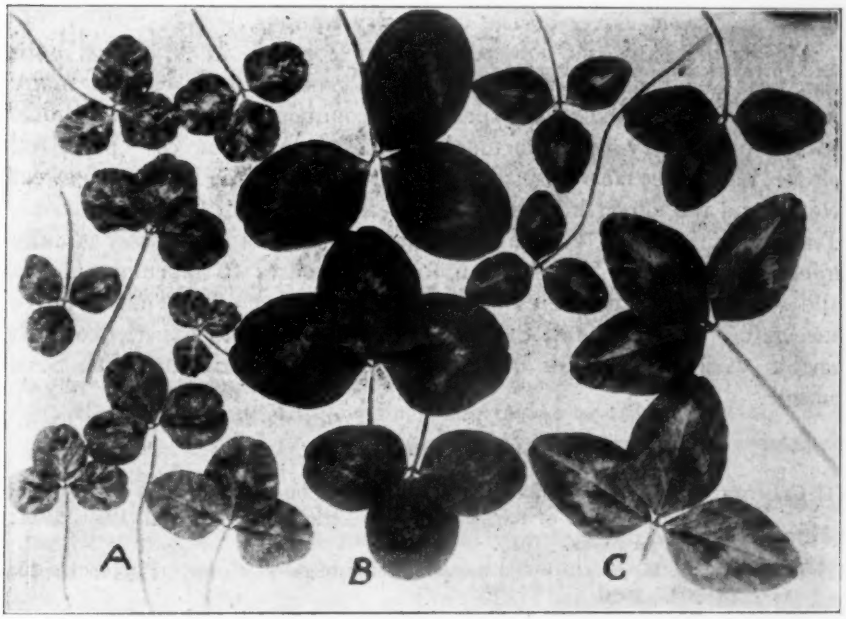

FIG. 1. *A*. Mosaic on red clover leaves. Natural infection. *B*. Healthy red clover leaves. *C*. Mosaic on red clover leaves about fifteen days after artificial inoculation.

[1] Common beans and cow peas were also planted but were destroyed by mice.

attempts at artificial infection and after all of the susceptible plants around them had become diseased either through artificial or natural inoculation. The disease appeared to be extremely virulent on the spotted burr clover.

A large red clover plant which had been used as one of the sources of the inoculum was allowed to grow in the greenhouse throughout the winter. In the spring it was noted that all signs of mosaic had disappeared from this plant, although the disease had remained throughout the winter on the sweet clover, horse bean, and burr clover. Crushed leaves from this plant failed to inoculate healthy red clover and sweet clover plants, but it was again inoculated from a burr clover plant. Careful observations were made in the field when the red clover and sweet clover began to grow in the spring of 1920. Mosaic appeared on very young shoots of sweet clover when it could not be found on red clover. The disease began to appear on the red clover somewhat later. It was thought possible that the disease was carried over the winter only on the sweet clover, but in an orchard in which red clover was abundant and in which no sweet clover was observed, the disease appeared approximately as early as on red clover in the mixed growth of red and sweet clover on the University campus.

Mosaic of beans and cow peas is very common in Arkansas, both cow peas and beans showing an extreme type of the disease in many instances. The inoculation experiments conducted would seem to point to the probability that the mosaic diseases on beans, cow peas, red clover, and sweet clover are identical, since the mosaic from both sweet clover and red clover infected four different genera of the leguminosae, Trifolium, Melilotus, Vicia and Medicago, and was apparently equally infectious on each. The reason for the failures to inoculate alfalfa and white clover are not evident, possibly further efforts might prove successful. Reddick and Stewart (3) had the same experience with certain varieties of beans in their inoculation experiments with bean mosaic.

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(3) REDDICK, D., and STEWART, V. B. Varieties of beans susceptible to mosaic. Phytopath. **8**: 530–535. Oct., 1918.

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A MODIFICATION OF THE CONCENTRATED FORMALDEHYDE METHOD OF SEED TREATMENT

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The concentrated formaldehyde method of seed treatment as proposed by Haskell[1] for the control of the smuts of oats, has many advantages over the formaldehyde dip or sprinkle methods and has recently become very popular in various parts of the United States. It seems to have proven quite uniformly successful wherever thoroughly tested in controlling the smuts of oats. This method calls for the application of a solution containing one part of 40 per cent formaldehyde and one part of water to the grain by means of a hand sprayer. The solution is to be applied at the rate of one quart to fifty bushels of oats. It has been considered that one stroke of the piston of the sprayer would deliver enough of the solution for each shovelful of grain.

It has been the experience of the writer that it is very difficult to apply uniformly so small an amount of solution to such a large amount of grain. This is especially true as the common quart sprayers vary greatly in the amount of solution which they will deliver at a single stroke of the piston. Another very serious objection to the method as proposed by Haskell has been that the fumes of such a strong solution of formaldehyde are very irritating to the mucus membrane of the nose and throat. Even when the grain is treated in the open air there has been more or less inconvenience experienced from this source.

In order to overcome these difficulties the writer has for the last three years thoroughly tested a modification of this method which has proven very satisfactory. Instead of equal parts of formaldehyde and water, ten parts of water to one of formaldehyde have been used. This makes a larger amount of the solution and it is therefore much easier to apply evenly to a given amount of seed. This modification moreover still retains the advantages of the "dry" method in that the grain is not wet sufficiently to necessitate drying. The fumes of the formaldehyde are not noticeable when this weaker solution is used. It is possible indeed to apply the solution in a closed room and not experience any inconvenience.

As stated above this modified method has been thoroughly tested for three years. Perfect control has been secured in every instance both in experimental plots and when the method has been used by farmers on a field basis. In 1918, at Corvallis, Oregon, badly smutted oats were treated with formaldehyde according to the standard dip method, the concentrated formaldehyde method, and the modified method as

[1] Haskell, R. J. The spray method of applying concentrated formaldehyde solution in the control of oat smut. Phytopath. **7:** 381–383. 1917.

outlined above. All three methods gave perfect control altho the checks were heavily smutted. In 1919 and again in 1920 at Moscow, Idaho, all three methods were again tested. In 1919 three lots of smutted oats were used with each treatment. These gave 12, 3 and 2 per cent smut respectively when untreated seed was planted. Perfect control was secured with each treatment except that lot number 1, which gave 12 per cent smut when untreated, gave 1 per cent when treated by the concentrated formaldehyde method.

In 1920 badly smutted Swedish Select oats when untreated gave 30 per cent smut while the same oats treated with the modified method as outlined above gave perfect control.

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A NOTE ON THE CORROSIVE SUBLIMATE TREATMENT FOR THE CONTROL OF RHIZOCTONIA

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Early in the spring of 1918, in connection with certain investigations on potato seed treatments being conducted at the University of Nebraska, the writer had occasion to make numerous plate cultures of Rhizoctonia from tubers treated with $HgCl_2$ solution at varying strengths and for varying lengths of time. In view of the recent studies of Melhus and Gilman[1] it has seemed advisable to present this data in brief form.

The general method of procedure was as follows: tubers bearing numerous sclerotia were soaked in corrosive sublimate, then carefully rinsed in sterile water. The sclerotia were picked off with a sterile scalpel and transferred to plates of potato agar. The plates were kept three days at a temperature of 27° to 30° C. after which the number of sclerotia that were growing were counted. The results are presented in the following table. It is noticeable that much better control was indicated in the shorter treatments with 1–1000 $HgCl_2$ and also with 1–2000 $HgCl_2$ than that reported by Melhus and Gilman. At dilutions greater than 1–2000 it was noticed that many sclerotia started to grow from the center, though the exterior was apparently killed by the fungicide. This was also noticed by Melhus and Gilman and described by them as the antiseptic rather than disinfectant effect of the fungicide. Unfortunately lack of available land did not permit the checking of these results under field conditions.

[1] Melhus, I. E. and Gilman, J. C. Measuring certain variable factors in potato seed treatment experiments. Phytopath. **11**: 6–17. 1921.

TABLE 1

Action of $HgCl_2$ on Sclerotia of Rhizoctonia

DILUTION	TIME	NO. SCLEROTIA PLANTED	PER CENT SCLEROTIA GREW
Checks (water)	60 min.	190	79
1–1000	5 "	60	0
1–1000	30 "	130	0
1–1000	120 "	100	0
1–1500	5 "	40	50
1–1500	30 "	40	0
1–1500	120 "	50	0
1–2000	5 "	60	66
1–2000	30 "	140	7.1
1–2000	120 "	80	0
1–2500	5 "	50	20
1–2500	30 "	60	5
1–2500	120 "	50	0
1–3000	5 "	50	80
1–3000	30 "	140	16
1–3000	120 "	90	22
1–4000	30 "	90	10
1–4000	120 "	30	0
1–5000	30 "	80	12
1–5000	120 "	40	0
1–6000	30 "	120	18
1–6000	120 "	90	11
1–7000	30 "	70	14
1–7000	120 "	40	2
1–8000	30 "	90	11
1–8000	120 "	60	6
1–9000	30 "	110	18
1–9000	120 "	40	25
1–10000	30 "	100	60
1–10000	120 "	40	30

PENNSYLVANIA STATE COLLEGE

FIELD CULTURES OF WOOD-ROTTING FUNGI ON AGARS

BESSIE E. ETTER

This article gives the apparatus needed for field inoculations on agar, method of packing the culture media for shipment, methods of inoculation, and the results obtained from the inoculations.

Many laboratories and schools have built culture rooms or inoculation chambers on the theory that such rooms were necessary if pure cultures

on artificial media were to be obtained; and text books on plant diseases usually recommend some form of culture room for such work.[1] In an article by Long and Harsch,[2] attention was called to the purity of both initial and sub-cultures, when made in an ordinary room without the use of any special inoculation chamber. In this case the purity of the cultures was attributed to the very dry climate of Albuquerque.

The data given in this article show that with proper care in making the inoculations, a high percentage of pure cultures can be obtained without the use of any culture room in moist or even wet climates, as well as in a dry climate.

The inoculations here reported were made in the field, without the use of inoculating chambers of any kind, in the following states: Kentucky, Mississippi, Louisiana, Florida, New Mexico, Arizona, Tennessee, and California, and in towns ranging in size from mere villages of one thousand population to cities of over three hundred thousand.

In New Orleans, the cultures were made during very foggy weather, and yet in spite of the moisture and the large amount of soot, and other foreign bodies in the air, the cultures were comparatively free from external contamination. Similar results were obtained at all the various places where the inoculations were made.

APPARATUS NEEDED FOR FIELD INOCULATIONS

The apparatus needed for field inoculations of wood-rotting fungi is neither complicated nor large in amount, as is shown by the following list: alcohol lamp, long-handled scissors, inoculating needles, scalpels, mallet, hatchet, saw, labels, alcohol, and a bottle containing alcohol for holding the scalpel and scissors when the actual inoculating is being done.

METHOD OF PACKING THE CULTURE MEDIA

Proper packing of the media is one of the most important features for the success of field inoculations since the culture media must be in such shape that it can be transported from place to place without danger of contamination or serious breakage. Both of these difficulties were avoided by using strong wooden boxes with hinged and padlocked tops, in which were packed heavy pasteboard boxes holding 72 test tubes each.

Necessary ventilation was obtained by boring 5 holes, one-half inch in diameter, in two sides of each wooden box. The paste-board boxes

[1] Smith, Erwin F. Bacteria in Relation to Plant Diseases, *485 p.* 1905, and Duggar, B. M. Fungous Diseases of Plants, *508 p.* 1909.

[2] Long, W. H. and Harsch, R. M., Pure cultures of wood-rotting fungi on artificial media, Jour. Agric. Res. **12:** 33–82. 1918.

held 6 layers of 12 test tubes each. These layers were separated from each other by corrugated paste-board, while the test tubes were separated by strips of the same material. The paste-board boxes were lined with currugated paste-board throughout and were an inch and a half wider than the length of the test tubes, thereby giving ample space for ventilation in each box. If the paste-board and wooden boxes are air tight, moisture will collect on the plugs in the test tubes and contamination, especially with molds, may result. The essential part in packing each paste-board box to prevent breakage in transit is to have the box so full that when its top is put on the tubes will be firmly held in place. The paste-board boxes should be securely tied with strong twine and fitted very tightly into the wooden boxes. It was found very convenient to pack an equal number of corn meal and malt agar tubes in each paste-board box, since 5 corn meal and 5 malt were used for each set of inoculations. Forty-two hundred test tubes, packed as above described were used in the field work discussed in this paper, and not a single test tube was contaminated in transit and only two were broken, in spite of the fact that some of these boxes of tubes were shipped from headquarters at Albuquerque, as far east as Florida, and as far west as California.

MEDIA USED

Corn meal agar and malt agar gave the best results for initial inoculations. Hard agars, say at least 3 per cent, adjusted to plus 1 Fuller's scale, in test tubes 20 by 150 millimeters, filled about two-thirds full and slanted were found best suited for field work. If the agar is not hard, the jarring incident to the shipping from place to place will cause it to break up and it then becomes unsuited for cultures.

METHODS OF INOCULATION

Any room closed sufficiently to prevent pronounced air currents proved satisfactory for inoculation purposes. All the inoculations were made on tables which had been freed from dust by a damp cloth, then covered with a freshly laundered towel. The scalpel and scissors were kept, during the period of inoculation, (when not in actual use) in a bottle of alcohol, and flamed red hot over an alcohol lamp before each inoculation. The inocula used consisted of pieces of diseased wood or of sporophores, which were carefully selected so as to be as free from molds and bacteria as possible. The diseased wood or sporophores were then split and the inocula taken from near the center of the freshly exposed surface. In making cultures from perennial sporophores the writer used with great success sections of the tubes of such species as

Fomes and Ganoderma for inocula. Pieces 2 by 2 by 5 millimeters were found more satisfactory than either larger or smaller pieces. The inocula should be inserted in the agar so as to leave at least one third of the piece exposed.

RESULTS OF INOCULATIONS

In the Mississippi Valley and Atlantic Coast States 240 sets of cultures were made, including 16 genera of fungi as follows: Armillaria, Corticium, Daedalea, Fomitiporia, Fomes, Ganoderma, Hydnum, Lentinus, Lenzites, Marasmius, Pholiota, Pleurotus, Polystictus, Polyporus, Poria, and Stereum also two unknown heart-rots. Of the 2400 tubes inoculated 590 showed contamination. The contamination in 577 of these tubes, unquestionably was already in the inocula. This leaves 13 tubes out of the 2400 which were contaminated by spores from the air.

Eighteen hundred test tubes were inoculated on the western trip. 12 genera were represented in these inoculations as follows: Cenangium, Fomes, Ganoderma, Irpex, Lentinus, Lenzites, Polyporus, Polystictus, Poria, Pleurotus, Stereum, and Trametes. 121 (less than 7 per cent) of these 1800 tubes showed contamination. Of this number only 4 showed contamination from the air.

The results obtained from both the eastern and western cultures show contamination from external sources as follows: 13 tubes out of 2400 (eastern) and 4 out of 1800 (western), or 17 out of 4200 test tubes inoculated, making an average of only 0.4 per cent contamination. This shows that cultures of wood-rotting fungi on artificial media can be satisfactorily performed in the field under varied climatic conditions if the precautions outlined in this article are followed.

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PHYTOPATHOLOGICAL NOTES

Symposium on dusting at the Chicago meeting. The American Phytopathological Society met in joint session with the American Association of Economic Entomologists at Chicago on December 21, 1920, for a program covering the subject, "Dusting as a Means of Controlling Injurious Insects and Plant Diseases." There were three papers on insect control and two papers on plant disease control. Prof. P. J. Parrott of Geneva, N. Y., reported the results of experiments on control of sucking insects by dust mixture, especially apple aphids, red bugs, currant aphids, and pear Psylla. Dr. T. J. Headlee of New Brunswick, N. J., presented data in regard to dusting as a means of controlling sucking and biting insects, with special reference to the plum curculio and the codling moth. Dr. A. L. Quaintance of Washington, D. C., reviewed some of the work of the U. S. Department of Agriculture on dusting vs. spraying of apples. Dr. H. A. Edson of Washington, D. C. gave a summary of the disease control work as related to truck crops. Dr. N. J. Giddings presented a general review of the investigations regarding orchard disease control.

The five papers, with the discussion which followed their presentation, will appear in the April number of the Journal of Economic Entomology where they will be accessible to pathologists interested in the subject of dusting.—N. J. Giddings.

Recognition of scientific accomplishments. On January 6, 1921, at a "Utility Corn Show" held at Galesburg, Illinois, Mr. J. R. Holbert, Agrononomist, Office of Cereal Investigations, U. S. Department of Agriculture, received highly merited recognition at the hands of Illinois farm advisers and corn breeders. Mr. Eugene D. Funk, chairman, presented Mr. Holbert with a beautiful silver loving cup enscribed as follows: "Awarded to J. R. Holbert in recognition of unselfish devotion to study of corn diseases," it being recognized that Mr. Holbert has done much in developing, in Illinois, the smoother types of corn which are proving more resistant to the root and stalk rots. These smoother types have been found to produce higher and more satisfactory yields than the rougher types encouraged by the old score card. Accordingly, a new and specially prepared score card was used in the Galesburg Utility Corn Show.—A. G. Johnson.

Phytopathological research in Russia. For the last four or five years we have received not a single phytopathological paper from Russia. We have had no information even as to whether work on plant diseases was being carried on, and if so in what institutions and under what direction. We now learn at least that phytopathological research has been preserved and apparently there is good prospect that it may be given additional opportunity for growth and development. The Slavic Division of the Library of Congress, Washington, D. C., is in receipt of a copy of an official journal of the Commissariat of Agriculture of the R. S. F. S. R.* (Vestnik Narodnogo Komissariata Zemledelia, No. 1, January, 1919). It consists of 90 pages and contains articles relative to the agricultural policies of the government and semi-popular articles on various topics. Among these there is one entitled "The Status of the Experimental Division in the System of Organization of the Commissariat of Agriculture" (pp. 55–59). From this it appears that the organization and conduct of the major part of the experimental work in Russia is to be supervised and facilitated by an office of the Commissariat, known as the Experimental Division. Enumerating various categories of investigational activities which are placed in the jurisdiction of the latter, the article refers to plant pathology in the following paragraph: "8. Study of enemies of agricultural plants, of orchard, garden, and vineyard and working out means of combatting them (entomological and phytopathological investigations and measures of combatting animal and plant parasites)." The number also contains announcements of several other agricultural journals and of a series of pamphets, circulars, and colored posters. It is to be hoped that the Library of Congress may be successful in procuring additional Russian publications from which we may learn more about phytopathological research in Russia.—M. SHAPOVALOV.

* Russian Socialist Federal Soviet Republic.